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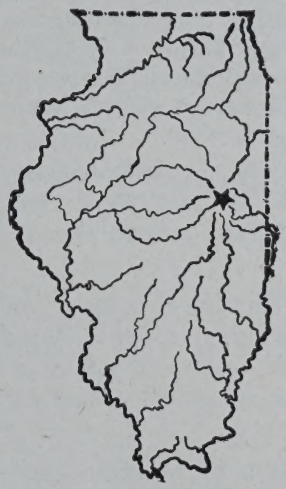
FATE OF TUBERCLE BACILLI OUTSIDE THE ANIMAL BODY

By CHAS. F. BRISCOE



URBANA, ILLINOIS, NOVEMBER, 1912

Letter of Transmittal

University of Illinois, June, 1912.

Dr. Eugene Davenport,
Director of the Agricultural Experiment Station.

Sir: I have the honor to transmit and recommend for publication the accompanying paper prepared under my direction by Chas. F. Briscoe, Ph.D., upon the Fate of Tubercle Bacilli Outside the Animal Body.

This is a companion bulletin with No. 149 of the University of Illinois Agricultural Experiment Station issued under date of February, 1911, entitled Tuberculosis of Farm Animals, by Chas. F. Briscoe and W. J. MacNeal. That bulletin was based upon a general study of the disease in bovine animals—its characteristics, modes of dissemination and infection, methods of recognition and prevention, and the relation to tuberculosis in man.

The import of the present paper is well expressed in the title, with the understanding that the studies were made principally upon the bovine type of the causative organism. Much previous work had been done upon the same subject, as is indicated in the literature herein cited, but important contributions to existent knowledge are made in the results of the experiments embodied in this publication, while earlier announcements have been confirmed or disproved. The whole matter is of such tremendous significance that any additions to knowledge and any further dissemination of knowledge upon the subject is of wide and vital importance. The information here presented will be welcomed by specialists, sanitarians, and the general public.

T. J. Burrill,
Chief in Botany (including Bacteriology).

1. There are four recognized types of tubercle bacilli; human, bovine, avian, and a type that infects cold-blooded animals. Only the first two types have any important part in the infection of man. Page 284

2. The beaded appearance of these germs led the early investigators to a belief in spore-formation. This is now known not to occur. The fatty content of the bacilli varies from 10 to 42 percent, which is five times as much as found in any other microorganism. It appears that this fatty material has little or nothing to do with the duration of their viability. Page 284

3. The tubercle bacillus does not secrete a soluble toxin, but that poisons are formed is well known. It has been shown by various investigators that tubercles can be produced in test animals by the injection of dead cultures. Tubercles thus produced may be mistaken for those produced by living germs. Page 284

4. A clear criterion of death is necessary in reporting results on the duration of life of the tubercle bacillus. This fact many investigators have disregarded. Page 285

5. The tubercle bacillus may be classed with the nonspore-bearing organisms as to viability; but in this class it is one of the most resistant, especially as to drying and to the antagonism of decay organisms in water and foul matter. Page 286

6. The action of sunlight upon bacteria was first studied by Downes and Blunt in 1877. Numerous investigators have continued this study to the present day. One of the most important facts brought out is that bacteria when directly exposed to the sun are killed in a few minutes. This is due to the effect of the ultra violet light. The ultra violet rays are now cheaply produced artificially by the mercury vapor lamp. This lamp is destined to play an important part in sterilization and disinfection. Pages 287-291

7. It is shown by the results of all the investigators given in Table 2 that tubercle bacilli when exposed directly to the sun are killed in a few minutes to a few hours. The time of killing is less at higher altitudes; but it is ten to fifteen times longer in diffuse light. Pages 292-295

8. Tuberculous sputum reduced to dust and inhaled by test animals causes tuberculosis. A much less amount is necessary to produce tuberculosis by inhalation than by ingestion. This, how-

ever, must not be taken to indicate that inhalation plays a more important part than ingestion as a cause of tuberculosis. The more important method depends upon the opportunity of infection from each. Investigators do not agree on this question. At present both inhalation and ingestion should be considered dangerous sources of tuberculous infection. Pages 295-306

9. The Mills-Reincke phenomenon, which has been given a mathematical equivalent by Hazen's theorem, viz., "Where one death from typhoid fever has been avoided by the use of better water, a certain number of deaths, probably two or three, from other causes have been avoided," has been found by Sedgwick and MacNutt to be sound and conservative. Their studies show that tuberculosis has decreased in certain cities of Massachusetts, which decrease is evidently due, in part, to the improvement of their water supply. Pages 307-308

10. It is reported in the literature that tubercle bacilli live for a very long time, several months to more than a year, in water and other material. Pages 309-310

11. In experiments to determine the time that tubercle bacilli live in various conditions the chief difficulty is the "index of death" for these germs. This is true since cultivation of the tubercle bacilli from contaminated material is not feasible, and since the dead germs produce, in test animals, tubercles indistinguishable by microscopic appearance from those produced by live tubercle bacilli. Pages 311-313

12. Pure cultures of nonspore-bearing organisms and the vegetative cells of spore-bearing germs when exposed to direct sunlight in thin smears are killed in ½ to 6 minutes; the human, bovine, and avian types of tubercle bacilli exposed in the same way were killed in 1 to 4 minutes. Pages 314-317

13. When exposed to desiccation in a dark, well-ventilated place, the nonspore-bearing organisms and the vegetative cells of spore-bearing organisms died in 1 to 4 days; spores of *B. subtilis* and *B. vulgatus* used as controls were not killed in 35 days; the human and bovine type of tubercle bacilli exposed at the same time and under the same conditions were dead within 4 and 8 days respectively. Pages 317-318

14. Pure cultures of bovine tubercle bacilli mixed in cow manure and exposed in a two-inch layer in a pasture field in the sunshine remained alive and virulent for two months. Pages 323-324

15. As would be expected, these germs exposed in cow manure retained their virulence longer in the shade than in the sunshine, as shown both by the greater severity of the disease produced in the guinea pigs inoculated with the germs exposed in the shade, than that produced in the guinea pigs inoculated on the same day with the germs exposed in the sunshine, and by the greater length of time that the guinea pigs which were inoculated with the germs exposed in the sunshine remained alive. Pages 325-326

16. Tubercle bacilli in the manure of a naturally infected cow exposed in the same manner as the artificially infected manure were dead within two weeks after exposure. Pages 327-334

17. Tubercle bacilli in garden soil and in a dead tuberculous guinea pig buried in garden soil were alive on the 213th and the 71st days, respectively, and dead on the 230th and 99th days, after first exposed. Pages 334-339

18. Tubercle bacilli live for more than a year in running water. A watering trough harboring these germs may be a dangerous source of infection to cattle. The better disposition of dead tuberculous animals is to destroy by burning. Tubercle bacilli in drinking water is one of the possible sources of infection for man. Infection is not prevented by dilution, since clumps containing a great number of these organisms may be inclosed in mucoid material which prevents their separation and destruction. Pages 340-359

19. Tubercle bacilli in market butter placed in cold storage live for more than ten months, which is a longer time than such butter is usually kept in storage. Pages 359-363

20. General discussion. Pages 364-365

21. References. Page 366

FATE OF TUBERCLE BACILLI OUTSIDE THE ANIMAL BODY

By CHAS. F. BRISCOE, Instructor in Botany[1]

INTRODUCTION

The fact that one-tenth of all deaths in the human family are due to tuberculosis, and that millions of dollars worth of farm animals are lost annually from its ravages, makes any knowledge concerning the fate of tubercle bacilli outside of the animal body of great value.

These questions have often come from stock owners: How long is it necessary to keep healthy stock from a field where tuberculous cattle have been previously allowed to run? How long do tubercle bacilli live in manure, in a watering trough, and in a dead tuberculous animal? And again, some authors have questioned the reports in the literature that tubercle bacilli remain alive and virulent for periods of a year and more outside of the animal body. They have suspected that in such cases the tubercles, found in the test animals after inoculation with such tuberculous material, kept for these long periods, had been produced by dead tubercle bacilli. It has been, in part, the purpose of this bulletin to answer these questions.

The first section is devoted to brief notes on the biology of the tubercle bacillus; then a tabular review of the literature is given with brief discussions; and lastly an account of the experimental work, followed by a general discussion. The experiments deal with pure cultures of tubercle bacilli, and with those of some other bacteria for purposes of comparison as to their duration of life in sunlight and under desiccation; and with the time limit of life of tubercle bacilli in cow manure, garden soil, water, butter, and dead tuberculous animals.

BIOLOGY OF THE TUBERCLE BACILLUS

MORPHOLOGY The tubercle bacillus varies in form according to type, method of growth, and age of the individual culture. It is a slightly curved, rod-shaped organism measuring from 0.3 to 0.5 microns in diameter and from 2 to 5 microns in

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length. There are two well-recognized types that infect mammals, the human and the bovine. The latter is shorter and thicker and stains more uniformly. The avian type, found in domestic fowls and birds, and a type that infects the cold-blooded animals, especially fishes and frogs, play almost no part in the infection of man.

The tubercle bacillus often presents a beaded appearance. This is more common in the specimens found in old pus and sputum. It is due to a fragmentation of the protoplasm. This peculiar structure of the organism led the earlier scientists to the belief in spore formation. Koch[74], in his first paper, *Die Aetiology der Tuberculos,* held to this belief, and this idea of spore formation has found place in many publications even to the present day. It is now known, from the relation of these organisms to the action of heat, sunshine and chemical disinfectants, that they do not form spores. They are killed very readily, in thin layers, by the direct rays of the sun, and also at a temperature of 60° C. in fifteen to twenty minutes. In these respects they are like other nonspore-bearing organisms.

WAXY OR FATTY SUBSTANCE

Their power of resistance to drying and to the antagonism of decay organisms appears to be greater than that of other nonspore-bearing organisms, tho less than that of the spore organisms. This power of resistance is no doubt due, in part at least, to the content of waxy or fatty substance found largely in the outer layer of the tubercle bacillus. The presence of this waxy material gives them their well-known character of "acid proof" power when stained. (When this waxy or fatty substance is extracted with ether, stains are no longer held on treatment with an acid or alcohol.) The bacillus has the largest amount of fatty substance of any known micro-organism. The fat content varies, according to different investigators, from 10 to 42 percent; while in other micro-organisms an alcohol ether-extractive has been found to vary only from 1.7 to 10.1 percent.

POISONS OF THE TUBERCLE BACILLUS

The tubercle bacillus does not secrete a soluble toxin, as do *B. diphtheria* and *B. tetani.* It has not been demonstrated that the tubercle bacillus forms a true toxin. Levene[87] proved the absence of tox-albumins from extracts of the bacillus. That poisons are formed is well known, tho their character is not understood. Baldwin[10] thinks that the symptoms and toxemia of tuberculosis are accounted for by the presence of the nucleic acid products in the blood. Koch[73], Von Prudden and Hodenpyle[155], Vissman[154], Straus and Gamaleia[144], Sternberg[145], Krompecher[75], Miller[97], and Rosenau[121]

have produced tuberculosis in test animals (guinea pigs and rabbits) by injecting dead cultures. These results have been confirmed in the laboratory here. (See experiments page 312).

LESIONS FROM DEAD CULTURES

The injection of dead cultures killed by heat or by chemical disinfectants will produce necrosis, abscesses, caseation, emaciation, and death. So closely do these lesions resemble those produced by the living germs that it is difficult to know whether we are dealing with dead or with living cultures. Rosenau[123] suggests that much of the work done upon the duration of life of tubercle bacilli has little value for lack of a clear criterion of death. Lesions from dead cultures, as from live ones, may be characterized by giant cells. Tuberculin cannot be depended upon to distinguish between the lesions produced by dead tubercle bacilli and those produced by live germs. The reactions caused by the injection of 2 cc. of tuberculin under the skin of guinea pigs with lesions produced by dead cultures are similar to those caused by a like injection into guinea pigs with lesions produced from living cultures, even to the death of the guinea pig.

Most investigators have not taken into account the fact that dead tubercle bacilli produce lesions, and in reporting their results have taken for granted, when at autopsy even local lesions have been found, that the tubercle bacilli have been alive and virulent. The only safe method of distinguishing is by inoculating a secondary healthy guinea pig with a small amount of the tuberculous material from the lesions of the original test animal. If the bacteria are alive, there develops a generalized tuberculosis, usually severe; if dead, either no lesions at all or only a slight localized effect will occur. Cultivations from this tuberculous material from both the original and the secondary test animal will give further evidence. If the organisms are dead, no growth occurs; if alive, growth is usually evident. This method, tho time consuming, is necessary to obtain the most trustworthy information.

RELATION OF TUBERCLE BACILLI TO OTHER ORGANISMS

That the tubercle bacillus does not possess the resistance to external agents as do the spore-bearing organisms is unquestioned. The spores of *Bact. anthracis* have been known to hold their virulence from ten to twelve years (Aiello and Drago[2]). We have found that the spores of *B. subtilis* dried on an agar slant and remaining in this state for eight years, gave growth when seeded into broth. It might be expected, however, that tubercle bacilli, from their waxy-fatty content and from their analogy to spores in respect to staining qualities, would be more resistant to

external injurious agencies such as heat, drying, light, chemicals, and putrefaction. This, in general, is found not to be the case. Rosenau[123] says, "The tubercle bacillus may be classed with the nonspore-bearing organisms so far as its viability is concerned." This statement is surely correct with respect to heat and light. The thermal death point of the tubercle bacillus, as determined by the more careful investigators (Herr[56], 1901; Hess[57], 1901; Russell and Hastings [127], 1904; and Rosenau [121], 1908), is given at 60° C., with an exposure of from fifteen to twenty minutes. This is practically the same as the thermal death point determined for most other nonspore-bearing organisms. (Sternberg[145], Smith[136]).

As regards desiccation, and more especially the antagonism offered by decay and foul matter, tubercle bacilli appear rather to take an intermediate position between spore- and nonspore-bearers. Rickards, Slack and Arms[119] found that tubercle bacilli in sputum resisted drying for 88 days; LeNoir and Camus[83] found these organisms alive after 33 days; Kuss[77] found them alive from 20 to 30 days, and killed in 40 to 60 days; Noetel[105] found them yet living after 35 days. Maffucci[90] shows that pure cultures of avian tubercle bacilli dried on silk threads live for 14 days. Kirstein[69] finds pure cultures dried in dust living from 3 to 8 days. In my own experiments they lived 8 days. Other nonspore-bearing organisms die in a very short time, as *B. violaceus,* 1 day; *B. typhoid,* 3 days; and *B. coli,* 3 days. There are reported in the literature cases where nonspore-bearing organisms have resisted drying, under special conditions, for a very long time. Rosenau[123] finds *B. pestis* to live over four months on a piece of dry sponge. Sirena and Alessi[135] report that the pneumonia diplococcus, when dried on silk threads kept in a moist room, did not die until after 192 days. More comparative work must be done with cultures of different nonspore-bearers and with tubercle bacilli exposed under the same conditions before definite conclusions can be drawn.

In the presence of foul material tubercle bacilli live from a month to a year and more (see Table 7). In my own experiments they were always found to live in water for 202 days and as long as 441 days. In this work much care was taken to determine that the tuberculosis produced in the test animals was by living germs. Careful workers like Jordan, Russell and Zeit[65] have found *B. typhosus* living in sewage from 3 to 4 days only; Russell and Fuller[126] report from 8 to 10 days. It would be expected that tubercle bacilli protected by the mucoid material, as found in sputum and diseased tissues, in which these germs more frequently occur, and also by their abundance of naturally waxy constituents,

would be protected against drying and injuries from the presence of foul material. This conclusion is well borne out by the literature and by the experimental data given in this bulletin.

REVIEW AND DISCUSSION OF THE LITERATURE

THE ACTION OF SUNLIGHT ON BACTERIA

Historical

The killing power of sunlight on bacteria was first noted by Downes and Blunt[34] in 1877. They worked with mixed cultures suspended in culture solutions and found that the organisms were killed in a short time, as shown by the solutions remaining clear. Tyndall[150] questioned the results of Downes and Blunt but later published results[151] confirming them. Duclaux[37] (1879) was the first to test the action of sunlight upon pure cultures and thus place the work upon a scientific basis. Arloing[7] (1885) was the first to test the action of light upon pathogenic bacteria (*Bact. anthracis*). He was also the first to use the electric light in such experimental work. Feltz[38] (1890) was the first to test the action of light upon tubercle bacilli. The classic works of Buchner[16] and Ward[157] (1892-3) deserve special mention. Buchner was the first to exhibit the killing power of light in reproducing characters by the growth of the organisms in that part of the Petri dish protected from the sunlight. The organisms are thickly seeded in a solid medium like agar, and the desired characters cut from black paper are fastened over this Petri dish. The organisms exposed to the sunlight are killed; where protected by the black paper, they grow, forming the letters. Ward called especial attention to the effect of different rays of the sun, which he separated by the use of screens of colored glass and colored solutions.

There early arose the questions: To what is the killing of bacteria due? and, Are spores more easily killed than the vegetative cells? Arloing[7] (1885) showed that spores of *Bact. anthracis* in broth were killed in two hours, while to kill the vegetative cells required from twenty-six to thirty hours. Nocard[104] (1885) suggested that during the exposure the spores developed into bacilli and the light acted upon the more sensitive vegetative cells. Straus[141] (1886) apparently confirmed this suggestion of Nocard by exposing anthrax spores in broth and in distilled water. He found that the spores in the broth were killed in nine hours while those in distilled water were not killed in this time. In 1886 Arloing[8] repeated his experiments, exposing the cultures on ice so that the spores could not develop, and confirmed his earlier results. Roux[125] (1887) finds the explanation in the fact that spores are more readily killed by the action of sunlight in the presence of

oxygen than when the oxygen is excluded. He thinks that from the nature of the spore constituents more oxidation products are formed in the spore than in the vegetative cell, and that for this reason the spore is more readily killed. Dieudonne[31] (1894) concludes that the formation of hydrogen peroxid by the action of sunlight on the presence of oxygen forms an important part in the killing of bacteria. Kruse[76] (1895) and Richardson[118] (1893) also came to this same conclusion. Thiele and Wolf[147] (1906) carried out carefully planned experiments in which their cultures of *B. prodigiosus, B. pyocyaneus* and *B. coli* were exposed in the presence of air, oxygen, or hydrogen. They took special precautions to confine the gases used, with mercury joints, so as not to allow the slightest diffusion. They found that the bacteria were killed as readily anaerobically as aerobically, and conclude, therefore, that the killing of the bacteria by light is not assignable to the indirect influence of oxidation of water. They exposed their cultures in broth diluted 1-1000, physiological salt solution and Elbe river water.

It can readily be shown that by the exposure of physiological salt solution to sunlight for the length of time the investigators exposed their cultures there is formed some condition sufficient to kill bacteria. The question whether the bactericidal action is due to an injurious chemical formed by the action of the sunlight, or whether it is a direct bactericidal action of the light, is still unsettled. The facts thus far established indicate that the action is due to both a chemical and a direct bactericidal action, sometimes one and sometimes the other being predominant, depending upon the condition of the experiment. A more important fact, which has been especially emphasized in a recent publication (see Weinzirl, Table 2) is that bacteria when directly exposed to sunlight are killed in a few minutes, in contrast to most of the published results of a few hours to a few days. This is due to the well-known germicidal effect of the ultra violet rays. Since glass is an excellent screen for these rays, an exposure of bacteria in glass containers lengthens very greatly the time necessary to kill.

Powerful beams of ultra violet rays are now artificially produced with the quartz mercury vapor lamp. A number of these machines are upon the market. The rays from them are so effective that bacteria directly exposed in thin layers are killed almost instantly. Indeed, so strong are the most powerful of these lamps that the skin accidently exposed is killed and later sloughs off.

It is stated that the sterilization of a city water supply can be done economically with these lamps. A test[5] at Marseilles, France, shows that the cost per million gallons of water is only ten dollars,

i. e., one cent per 1000 gallons. This indicates a feasible system of supplying a city with a good, potable water from a contaminated source. Years ago it was suggested by Marshall Ward that the disinfection of cow sheds might well be done with electric lights. Tho this was thought fanatical at the time, it may be feasible with such light as the present powerful mercury vapor lamp produces. Why cannot the "toning up" effect that is to be felt in a room which has been exposed to the sunlight thruout the day be in a way brought about by exposing to one of these powerful mercury vapor lamps, for a few hours in the evening, such rooms as have no access to the sun, at the same time securing the great benefit that comes from disinfection by its powerful beams?

For further discussion of this most interesting subject we refer our readers to the recent numbers of the periodicals, more especially the chemical and engineering journals. The following table gives a brief review of the results found in the literature upon the action of sunlight on bacteria.

TABLE 1.—ACTION OF SUNLIGHT UPON BACTERIA

Author	Date	Conditions	Not killed	Killed
Downes and Blunt[35]	1877	Cultures in Pasteur solutions		5 to 6 hrs.
Duclaux[37]	1879	Tyrothrix filiformis spores		35 days
Duclaux[36]	1885	Tyrothrix scaber from milk:		
		A drop dried in an empty flask	14 days	60 days
		In broth culture vegetative cells		15 days
		Kept in the dark	3 yrs.	
Duclaux[36]	1885	Micrococci, young cultures veal broth:		
		In dark	over 1 yr.	
		In sunshine, May to June		40 days
		In sunshine, July		15 days
		In dried condition		8, 3 and even 2 days
Arloing[6]	1885	Bact. anthracis spores in broth	1 to 1½ hrs.	after 2 hrs.
		Bact. anthracis vegetative cells in broth	2 hrs.	26 to 30 hrs.
Straus[141]	1886	Bact. anthracis spores:		
		In broth		9 hrs.
		In distilled water	9 hrs.	
Arloing[8]	1886	Bact. anthracis spores in broth on ice 4° C.		5 hrs.
Downes[33]	1886	Bacteria in mixed cultures, diffuse light		5 days
Roux[125]	1887	Bact. anthracis spores:		
		Without air	83 hrs.	
		With air		29 to 54 hrs.

TABLE 1.—*Continued*

Author	Date	Conditions	Not killed	Killed
Pansini[108]	1889	B. violaceus, B. prodigiosus, B. pyocyaneus, Bact. anthracis, Msp. comma, Murisepticus, and Staph. pyogenes aureus:		
		Exposed to diffuse daylight, development hindered	24 to 48 hrs.	
		Exposed to sunshine in tube cultures		1 day.
		In hanging drop on needle		½ to 2½ hrs.
Laurent[80]	1890	Bacillus of Kiel on potato	1 to 3 hrs.	5 hrs.
Janowsky[62]	1890	B. typhosus in culture media		4 to 10 hrs.
Buchner[16]	1892	B. typhosus on agar plates		1 to 1½ hrs.
Buchner[15]	1893	B. typhosus, B. pyocananeus, and Msp. comma on plates 1.6 meters under water		4½ hrs.
Geisler[50]	1892	B. typhosus (almost all killed)		3 to 6 hrs.
Momont[100]	1892	"Asporegene anthrax" in phenol broth:		
		Dried and exposed in air		5 to 5½ hrs.
		Dried and exposed in vacuo		6½ hrs.
		In moist condition in the air		2½ hrs.
		In moist condition, no air		50 hrs.
		Dried anthrax spores in the air		100 hrs.
		Dried anthrax spores in vacuo		100 hrs.
		Moist anthrax spores in the air		44 hrs.
		Moist anthrax spores in vacuo		100 hrs.
Ward[157]	1893	Bact. anthracis spores on agar		4 to 10 hrs.
Frankland and Appleyard[42]	1893	Bact. anthracis spores in Thames river water:		
		In diffuse daylight [1]	6 mo.	
		In direct sunshine		151 hrs.
		Bact. anthracis vegetative cells:		
		In unsterilized water	56 hrs.	84 hrs.
		In sterilized water	84 hrs.	
Ledoux-Lebard[81]	1893	B. diphtheria in broth culture		few days
Ward[157]	1893	Organisms on agar in Petri dishes with quartz glass covers		2 to 6 hrs.
Ward and Cartwright[158]	1893	Bact. anthracis in unsterilized Thames river water	over 7 mo.	
d'Arsonval and Charrin[9]	1894	B. pyocyaneus on agar	3 to 6 hrs.	
Gaillard[45]	1894	B. typhosus in water		3 to 4 hrs.
Dieudonne[31]	1894	B. coli, B. typhosus, Bact. anthracis, B. prodigiosus, and B. fluorescens		½ to 2½ hrs.
		By electric arc light 900 candle-power.	5 hrs.	8 hrs.
		Bact. anthracis spores:		
		In the air		3½ hrs.
		Air excluded	3½ hrs.	
		B. tetani spores, air excluded	9 hrs.	
Kruse[76]	1895	Bact. anthracis spores in hanging drop	1½ hrs.	2 to 5 hrs.
		B. typhosus:		
		In broth, air present		2½ to 7 hrs.
		In broth, air replaced with hydrogen	7 hrs.	
		Bact. anthracis, diffuse light	(13 days	80 days)

[1] Tho the spores were not killed, they were so weakened that one cubic centimeter did not kill mice. A broth culture of the same in much smaller quantity killed mice.

TABLE 1.—*Continued.*

Author	Date	Conditions	Not killed	Killed
Beck and Schultz[12]	1896	Bacteria		short time
Gehrke[49]	1899	Bacteria:		
		In clear water		2 to 8 hrs.
		Agar cultures		6 hrs.
Kedzior[67]	1899	B. pyocyaneus, B. diphtheria, Msp. metchnikovy, and spores of Bact. anthracis, exposed on gelatin		1¼ to 3¼ hrs.
Jones[64]	1900	B. cartovorus, directly exposed	5 min.	10 min.
Mettler[95]	1904	Msp. comma, B. typhosus, and B. coli in broth culture		few hours
Huber[61]	1905	Streptococci and B. diphtheria in broth		2 to 3 hrs.
Thiele[147] and Wolf	1906	B. coli, and B. typhosus in diluted broth, Elbe river water, or physiological salt solution		9 hrs.
Weinzirl[160]	1906	Exposed directly to the sun in thin smears:		
		B. cholera suis, B. prodigiosus, B. typhosus		5 to 10 min.
		B. typhosus (repeated), B. dysenteria, B. prodigiosus (repeated), B. pyocyaneus		2 to 5 min.
		Msp. comma		2 to 6 min.
		B. diphtheria		5 min.
		Pus cocci		5 to 15 min.
		B. coli		½ to 2 min.
		B. friedländer		0 to 1 min.
		B. phosphorescens		1 to 2 min.
		Sar. aurantiaca		90 min.
		Pink air micrococci		120 min.
Neumark[103]	1907	Exposed to direct sunlight on agar plate:		
		B. anthracis spores		25 to 30 min.
		B. anthracis vegetative cells		20 to 25 min.
		B. coli		60 min.
		"Schweinpest bacilli"		110 to 120 min.
		Chicken cholera		8 to 10 min.
		Schwein erysipelas		240 min.
Orsi[106]	1907	B. typhosus and Msp. comma in sunlight		8 to 10 hrs.
		B. coli in distilled water	6 hrs.	12 hrs.
McNaught and Korich[94]	1910	B. coli:		
		In distilled water in air at 0° to 15°C. in diffuse daylight	25 days	
		In strong sunlight, numbers much decreased	6 hrs.	
		In strong sunlight		12 hrs.
		Dried in a desiccator	11 days	

THE DURATION OF LIFE OF TUBERCLE BACILLI IN SUNSHINE AND IN DIFFUSE DAYLIGHT

INDEX OF DEATH

Every author consulted, except Weinzirl, used the animal test to determine when tubercle bacilli were dead. Weinzirl, using only pure cultures, employed the method of cultivation. No investigator whose work is recorded in Tables 2 and 3 has called attention to the fact that dead cultures produce tuberculosis (for discussion see page 112). Just what error a neglect of attention to this point has introduced into the results given in the following table cannot be definitely known. Tho it is certain that dead tubercle bacilli do produce tuberculosis, yet in no case was it found in my own experimental work that localized tuberculosis was produced by dead organisms. In every case the secondary guinea pigs inoculated from cases of localized tuberculosis became tuberculous and usually severely infected. Cultures from diseased tissues produced growth, either from the original or from the secondary inoculated guinea pigs, or from both. It appears, therefore, that the error arising from not taking into account tuberculosis produced by dead cultures is probably slight.

TIME REQUIRED TO KILL

Pure cultures of tubercle bacilli when exposed in thin layers to the direct sunlight are killed in from a few minutes to a few hours. This has been the result obtained by all investigators. To kill these germs in sputum requires only a slightly longer time. The mucoid mass is a slight protection which increases with the thickness and opacity of the material, but even here tubercle bacilli are killed in a very short time. With two exceptions, that of Feltz[38] and of Mitchell and Crouch[98] (see Table 2), no investigator reports these bacilli surely living after twenty-four hours' exposure to the sun. In these two cases the sputum was exposed on soil, and a small layer of soil would afford good protection from the action of the sunlight.

EFFECT OF HIGH ALTITUDE

Experiments definitely planned to test the killing power of the sun on tubercle bacilli at different altitudes have been carried out by a Russian, Treskinskaja[148]. In our country Mitchell and Crouch[98] have determined the effect of the sun on tuberculous sputum at a height of 1356 meters. Treskinskaja used pure cultures emulsified in one-percent peptone solution, spread in thin layers and exposed directly to the sun. These were killed as follows: At a height of 1560 meters, in three hours; at 903 meters, in four hours; at sea level, in four hours. The peptone solution when dried gave a thin protective layer covering these organisms. Treskinskaja thought that this protection would be about equal to that of a thin layer of sputum.

The difference in killing power is due to the difference in ultra violet light. These rays are largely absorbed by the atmosphere, especially when laden with moisture. According to Langley, only 39 percent of the ultra violet light reaches the sea level. The higher the altitude, the dryer the atmosphere, the more intense is the light and the greater its killing power.

Mitchell and Crouch found that tubercle bacilli in sputum were not killed in 35 hours, but were killed in 45 hours. These experiments are not comparable with the experiments of Treskinskaja, done at nearly the same altitude, since the soil and sputum give far more protection than exposure in a thin layer on glass from a one-percent peptone solution.

DISCREPANCIES IN RESULTS It is not to be expected that tubercle bacilli under different conditions will be killed in the same length of time. It is indeed difficult to expose different pure cultures under the same conditions. There may be a difference in the age of the culture, in the uniformity of the emulsion, in the manner of exposing, and in the means of determining the time of killing the germs. When exposed on threads of linen or silk some of the germs may be well protected and live for a much longer time than when exposed in a thin layer on a glass slip or on sterilized glazed paper. Then if the material in which they are exposed, as sputum, is of such a nature as to give protection from drying as well as from the rays of the sun, the difference will be still greater. This accounts, partly at least, for the difference in the results of Sawitsky[128], which showed that tubercle bacilli in sputum on linen threads lived two and one-half months, and the fact reported by Weinzirl[160], that a pure culture in thin layers exposed directly to the sun was killed in a few minutes. It is not possible to give definite reasons for all the discrepancies found in the results shown in Table 2; but excepting those of Feltz[38], whose material mixed in the soil might well give complete protection from the sun, it will be seen that tubercle bacilli exposed to the sunlight are killed within a few minutes to a few hours.

DIFFUSE LIGHT It takes ten to fifteen times as long for tubercle bacilli to be killed in diffuse light as in direct sunlight; yet these germs are killed much sooner in diffuse daylight than in the dark, when under the same conditions otherwise, as may be seen by comparing the results of Rickards, Slack and Arms[119], Twichell[149], and Ransome and Delepine[114].

Tables 2 and 3, respectively, give in brief the literature upon these subjects.

TABLE 2.—EFFECT OF SUNSHINE ON TUBERCLE BACILLI

Author	Date	Conditions	Not killed	Killed
Feltz[38]	1890	Sputum and soil mixed and exposed to direct sunlight, tested by guinea pig inoculation	137 days	after 137 days
		Exposed to changing climatic conditions	2½ mo.	after 2½ mo.
Koch[73]	1890	In pure culture		few min. to few hrs.
Sawitsky[128]	1891	Sputum on stretched linen		2½ mo.
Ransome and Delepine[114]	1894	Sputum exposed to light		45 days
		Pure culture on glazed paper		4 days (12½ hrs. sun)
Renzi[117]	1894	Sputa mixed with 10 parts water, exposed at 28° C	6 hrs.	
Migneco[96]	1895	Sputum on stretched linen and woolen threads	10 to 15 hrs.	24 to 30 hrs.
Straus[142]	1895	Culture in a glass container, in broth		2 hrs.
		In a thick layer of dried sputum	1 to 12 hrs.	
Gardiner[47]	1898	In sputum	24 hrs.	
Ottolenghi[107]	1899	In sputum on woolen cloth		40 hrs.
		In sputum on linen cloth		24 to 30 hrs.
		In sputum on paper		9 hrs.
		Culture on paper		6 hrs.
	1900	Sputum the thickness of spitting, on paper		4 to 14½ hrs.
Jousset[66]	1900	Sputum exposed to direct sun	1 hr.	5 to 7 hrs.
		Another similar experiment		1 hr.
Mitchell and Crouch[98]	1900	Tuberculous sputum placed upon sterilized soil	35 hrs.[1]	45 hrs.
Abba and Barelli[1]	1901	Sputa on glass and wood		6 days
Annett[4]	1902	Tested 105 specimens of sputa taken at random; 5, or 4.76 percent contained tubercle bacilli	4.76 percent	
	1903	Tuberculous sputum size of spitting	2 to 24 hrs.	48 hrs.
Cadeac[18]	1905	Tuberculous sputum on a board		24 hrs.
		Tuberculous sputum on glass	24 hrs.	48 hrs.
Bang[11]	1905	Tubercle bacilli on agar, exposed to a 30-ampere lamp at 30 cm. distance		6 min.
Twitchell[149]	1905	Tuberculous sputum, direct rays	1 hr.	7 hrs.
Didonna[30]	1906	Exposed culture to sun 2 to 8 hours; then inoculated guinea pigs in order to immunize them. Produced only a local abcess	2 to 8 hrs.[2]	
Weinzirl[160]	1906	Pure culture on paper slips or glass covers		2 to 10 min.
Rickards, Slack and Arms[119]	1909	Sputum exposed in sunshine	6 hrs.	
Treskinskaja[148]	1910	Summer months, direct sun:		
		Pure culture tubercle bacilli, height 1560 m		3 hrs.
		Pure culture tubercle bacilli, 903 m		4 hrs.
		Pure culture tubercle bacilli, sea level		4 hrs.

[1] Virulence diminished after 20 hours.

[2] It is not certain whether the organisms were alive or dead, since dead organisms can produce tubercles.

TABLE 3.—ACTION OF DIFFUSE LIGHT UPON TUBERCLE BACILLI

Author	Date	Conditions	Not killed	Killed
Galtier[46]	1889	Tuberculous organs:		
		Dried at 30°	38 days	30 days
		Dried at room temperature	(some) 30 days	(usually)after 30 days
Sawitsky[128]	1892	Sputum on stretched linen		2½ mo.
Ransome and Delepine[114]	1894	Tubercle bacilli on dried paper		2 days
Lucibelli[89]	1899	Sputum dried on glass		18 days
		Fluid sputum in reagent glass		4 mo.
Jousset[66]	1900	Sputum, Series 1		4 to 7 hrs.
		Series 2	4 to 7 hrs.	
	1902	Sputum in fine drops on glass:		8 to 14 days
		Mixed with dust		8 days
		On threads	5 days	10 days
		On coarse cloth	20 days	
Hill[60]	1903	Sputum in reagent glass in glass cupboard		16 days
Cadeac[18]	1905	Sputum on glass	2 to 6 days	4 to 10 days
Twitchell[149]	1905	Tuberculous sputum in paraffined bottles	124 days	175 days
Sormani[138]	1906	Dry sputum in a room	1 mo.	
Rickards, Slack and Arms[119]	1908	Dry sputum in a room	1 mo.	
Weinzirl[159]	1908	Fine emulsion of tubercle bacilli dried on paper slips (average of ten trials)	2.8 days	4.4 days

EFFECT OF DESICCATION UPON TUBERCLE BACILLI IN SPUTUM AND OTHER MATERIAL

The difference in the length of time that different bacteria withstand desiccation is very great. *B. cartovorus* is killed in a few minutes to a few hours (Jones[64]); *B. tuberculosis* may be killed in a few days (14 days, Maffucci[90]) to several days (88 days, Rickards[119]); and spores of *Bact. anthracis* live twelve years (reported above).

The difference depends upon the kind of substance on which they are exposed and upon the difference in the kind and form of organism exposed. Harding and Prucha[54] have shown that *Bact. campestre* remains alive much longer when dried on cabbage seed than when dried on glass covers; on glass it was dead at the end of ten days; on the seed it remained alive for thirteen months. This difference is no doubt largely due to the difference in the hygroscopic moisture retained by these substances. The kind and form of the organism exposed to drying has even more to do with its capability of living. The spore form lives very much longer than the vegetative form.

It appears that tubercle bacilli, especially in sputum and other mucoid material, withstand desiccation better than other nonspore-formers. The difference is not great and there are many apparent exceptions where other nonspore-bearers live for a very long time, like the one given above after Harding and Prucha[54]. Diplococcus pneumonia has been reported as living for 192 days when dried on silk (Sirena and Alessi[135]).

The important fact which has been so thoroly established in recent years is that tuberculous sputum reduced to dust causes tuberculosis when experimental animals (guinea pigs, rabbits, cats, dogs and calves) are made to breathe such dust-laden air. How great the danger is to man and cattle to breathe dried tuberculous material is yet a disputed question. In 1882 Koch, in his work on "Die Aetiologie der Tuberculose", pointed out that dried tuberculous sputum is one of the most important factors in causing tuberculosis. It was not until 1905 when the work of Calmette and Guerin[21] was published that ingestion was brought forward as a very important factor in producing tuberculosis. It was suggested by the advocates of this theory that practically all tuberculosis is produced by ingestion—that even inspired material is swallowed and what apparently is inhalation tuberculosis is actually tuberculosis by ingestion. This conclusion will not hold in the light of the most recent experiments, especially those carried out at Breslau and collectively published by Flügge[40] (1908), "Verbreitungsweise and Bekämpfung der Tuberkulose". It is shown here that in all animals with which experiments have been made it required a hundred to a thousand times more tubercle bacilli to produce tuberculosis by feeding than it does by inhalation. So the thought that the swallowing of a part of the few tubercle bacilli necessary to produce tuberculosis by inhalation is the cause of ingestion tuberculosis is wholly precluded.

A complete discussion of this most interesting subject cannot be given here. The data in the two following tables show the fact mentioned above, that it requires many times more germs to produce tuberculosis by feeding than by inhalation; also another most important fact, that severe tuberculosis may be produced by either method of infection. It must not be understood that the facts exhibited in these two tables in any way indicate which is the more frequent method of infection. For what matter if it does require one thousand times more tubercle bacilli to produce tuberculosis by feeding than by inhalation, who knows whether we are, on the average, taking in one thousand times more of these germs in the food than in the breath?

On one hand, Calmette[19] is found declaring that "In the ordinary daily life, the infection of the digestive organs is predomi-

TABLE 4.—TUBERCULOSIS BY INHALING DEFINITE QUANTITIES OF TUBERCLE BACILLI

No.	Author	Date	Place	Test animal	Number	Amount inhaled, germs	Type	Result	Remarks
1	Findel[39]	1907	Breslau	Calf	1	120 million	Bovine	+	Germs inhaled in moist spray thru a tracheotomy tube.
				Dog 1	1	16.27 million	Bovine	+	Germs inhaled as above. All became severely tuberculous.
				Dog 2	1	9.80 million	Bovine	+	
				Dog 3	1	4.935 million	Bovine	+	
				Guinea pigs	65	290,000 to 20	Bovine	+	All except 3 of the 65 guinea pigs became tuberculous; two that had received 40 and one of the three that had received 20 individual germs remained healthy.
2	Heymann[58]	1908	Breslau	Guinea pigs	5	10,000	Human	+	The guinea pigs in each series were killed 1, 12, 24, 72 and 144 hours after inhalation.
				" "	5	100,000	Human	+	
				" "	5	1 million	Human	+	
3	Alexander[3]	1908	Breslau	Guinea pigs	16	50 to 500	Human sputum	+	All became tuberculous.
				Rabbits	14	100 to 50,000	Bovine	+	Uses the Findel tower apparatus for inhalation in the three series.
				Rabbits	10	5,000 to 50,000	Human	+	Only doses of 50,000 germs surely positive in the last series.
4	Reichenbach[116]	1908	Breslau	Guinea pigs	8	4,000 to 4 million	Human	+	The dose was given with the Buchner spray apparatus in the open air; so only a part was inhaled.
5	Kuss[77]	1908	France	Guinea pigs	9		Human	4+ 5−	Animals inhaled tuberculous sputum dried 12 to 13 days on a carpet; beaten and swept for 20 minutes.
				Guinea pigs	6		Human	2+ 4−	Same as above except dried for 6 days and beaten and swept for 12 minutes.
				Guinea pigs	9		Human	6+ 3−	Guinea pigs exposed during the time of the above two series and also the time of removing the first and placing the second series of test animals.

TABLE 5.—TUBERCULOSIS BY FEEDING DEFINITE QUANTITIES OF TUBERCLE BACILLI

No.	Author	Date	Place	Test animals	Number	Amount fed, germs	Type	Result	Remarks
1.	Calmette and Guerin[21]	1905	Lille	Young goats	2	40 mg.	Bovine	+	Suckled mothers whose teats were injected with pure culture of tubercle bacilli.
				Young goats	2	40 mg.	Human	+	Same as above.
				Young goats	3	200 mg.	Bovine	+	Fed thru a stomach tube.
				Young goats	2	100 mg. and 200 mg.	Human and Bovine	+	The human type of tubercle bacilli appeared not to injure the goats. In June they were given 200 mg. of bovine type.
				Adult goats	3	200 mg.	Bovine	+	All severely tuberculous; lung involvement.
2.	Calmette and Guerin[20]	1906	Lille	Cows	4	100, 250, 500 and 1000 mg. respectively	Bovine	+	All became tuberculous.
				Cows	6	250 mg.	Bovine	+	Six Breton heifers aged 8 to 10 months. All became tuberculous.
3.	Findel[39]	1907	Breslau	Dogs	5	172 to 4.48 mg.	Bovine	0	Three were fed at three intervals during 40 days; other two at one feeding.
				Guinea pigs	14	19,000 to 382,000	Bovine	0	Two control guinea pigs inoculated with 19,000 individual tubercle bacilli became severely tuberculous.
4.	Alexander[3]	1908	Breslau	Rabbits	24	5 to 180 mg.	Human	0	No tuberculosis in any animal.
				Rabbits	15	1 to 50 mg.	Bovine	0	Only slight evidence of tuberculosis in one that was fed 50 mg.
5.	Reichenbach[116]	1908	Breslau	Guinea pigs	8	4,000 to 4 million	Human	0	Not the slightest evidence of tuberculosis in any animal.
6.	McFadyean[93] (Griffith)	1910	London	Calves	22	1 to 5 million	Bovine	(0) +	Only one calf was completely negative. The presence of the disease was found 21 times in the abdominal organs, but only 3 times in the lungs. Always present in the abdominal organs when found in the lungs.

nant"; and on the other hand Chausse[23] says, "Tuberculosis in adult bovine is due to inhalation in 98 percent of the cases, if not more; in calves in about 90 percent, ingestion and congenital infection being responsible for the remainder." Dr. Ravenel[115], a strong believer in ingestion as one of the important causes of tuberculosis says, "One must take an impartial view of the whole problem, and be willing to agree that both channels of infection are open. In animals, however, the alimentary tract seems to be a more common port of entry".

At present, at least, both ways of infection should be considered very dangerous and every precaution taken to guard against them. A brief summary of the literature concerning the length of time that tubercle bacilli live in dried tuberculous material and concerning the infectiousness of such material is given in Table 6.

TABLE 6.—EFFECT OF DESICCATION OF TUBERCLE BACILLI IN SPUTUM AND OTHER MATERIAL

Author	Date	Conditions	Not killed	Killed
Villemin[153]	1869	Dried tuberculous sputum	several hrs.	
Koch[74]	1882	Dried tuberculous sputum	8 wks.	
Cochez[24]	1883	Tubercle bacilli incased in sputum	3 wks	
		Dried sputum killed a dog, by inhalation		
Malassez and Vignal[91]	1883	Alternate drying and moistening of tuberculous sputum 8 times	12 days	
Schill and Fischer[129]	1884	Tuberculous sputum with spores, dried on glass	126 days	179 days
		Tuberculous sputum without spores, dried on glass	186 days	226 days
DeThoma[29]	1886	Dried tuberculous sputum		10 mo.
Sormani[137]	1886	Dried tuberculous sputum	2 mo.	virulence deceased thereafter
		Tuberculous sputum dried on linen	6 mo.	no virulence after 4 mo.
Galtier[46]	1887	Dried tuberculous material	20 to 38 days	
Cadeac and Malet[17]	1888	Pieces of dried tuberculous lung exposed on paper in laboratory	43 days	102 days
		Dried tuberculous lung allowed to decompose in outer air	76 days	
		Same repeated	80 days	
		Same repeated	150 days	after 150 days
De Souza[28]	1888	Inhalation of dried tuberculous material caused tuberculosis in 12 of 14 guinea pigs. Time of drying not stated		
Galtier[46]	1888	Inoculation of tuberculous material dried at 30° C., or by breathing dried tuberculous material	15, 30 and 38 days	
		Same material dried at room temperature	30 days	after 30 days

TABLE 6.—*Continued*

Author	Date	Conditions	Not killed	Killed
Cornet[27]	1889	Dust samples examined for tubercle bacilli were found positive:		
		In hospitals	47.6 percent	
		In insane asylums	17.6 "	
		In dwelling houses	43.6 "	
Feltz[38]	1890	Dried tuberculous sputum in road dust:		
		Exposed to weather	over 7 mo.	
		Exposed to sun	about 140 days	
		Dried sputum, in rooms	7 to 9 mo.	
Sawitsky[128]	1891	Tuberculous sputum dried under ordinary conditions and exposed in the dark in living rooms	2½ mo.	
		Same exposed to sunlight	2½ mo.	
Stone[140]	1891	Dried tuberculous sputum, only decreased in virulence	3 yrs.	
Koch[72]	1892	Guinea pigs were inoculated with tuberculous sputum dried for 2 weeks, for 4 weeks, and for 8 weeks. In each case tuberculosis developed as with fresh material.	2 to 8 wks.	
Maffucci[90]	1892	Pure culture of avian tubercle bacill dried on silk threads, inoculated into a hen abdominally	14 days	
		Same repeated. Hen not tuberculous abdominally; one tubercle in the lung	2 mo. (?)	2 mo. (?)
Marpmann[92]	1893	Tubercle bacilli frequently found in street dust by microscopic test	(?)	
Ransome and Delepine[114]	1894	Tuberculous sputum exposed in watch glasses:		
		To air and light 4 days; then 15 days in the dark	19 days	
		To air and light 8 days; then 11 days in the dark		19 days
		In closed cupboard	19 days	
		In dark air shaft, produced slight tuberculosis in a rabbit	45 days	
Hance[53]	1895	The author, under the direction of Dr. Trudeau of the Adirondack Cottage Sanitarium, examined 81 samples of dust from the differ- cottages. Four guinea pigs died too early to make the test for tubercle bacilli; of those remaining 5, or 4.9 percent, became tuberculous. These were from a group of 10 guinea pigs inoculated with the dust from one small cottage. The author later inoculated 9 guinea pigs with dust from a New York hospital, 5 of which died in 3 days, and 1 of the 4 remaining became tuberculous	4.9 percent 25 percent	
Kirchner[68]	1895	Fourteen guinea pigs were inoculated with 7 samples of dust from rooms in homes of consumptives; 9 guinea pigs died early and 5 remained healthy. In a second ser-		all killed

TABLE 6.—*Continued*

Author	Date	Conditions	Not killed	Killed
Kirchner [68] (continued)		ies three rooms were investigated, from which 8 guinea pigs were inoculated; one died early and one of the other 7 became tuberculous.	14 percent	
Cornet [26]	1893	Tuberculous sputum placed on a carpet and allowed to dry naturally produced tuberculosis in 35 out of 36 guinea pigs which were placed in a room at a distance of 134 to 300 cm. from this carpet.....		
Laschtschenko [78]	1898	Tuberculous sputum dried in thick layers on paper and cloth was not virulent after 9 months.....		9 mo.
Neisser [102]	1898	Tuberculous sputum rubbed up with dust and aspirated at a velocity of 3 to 5 cm. per second will produce tuberculosis, when inoculated into guinea pigs, in a large majority of cases.....		
Beninde [13]	1899	Handkerchiefs that were used by consumptives from 2 to 12 hours were dried for 24 hours, and then rubbed up in a box. The dust from these was aspirated and injected into two guinea pigs for each test. In 12 tests 8 were positive and 4 negative.....	8 positive	4 negative
Cornet [26]	1899	Dried sputum in one case lost virulence in 3 months; another time remained virulent from 6 to 8 months.....	8 mo.	3 mo.
Sticker [139]	1899	Tested 29 samples of sputum dried from 2 to 21 days, by inoculating guinea pigs with dried sputum dust and by causing them to inhale the dried sputum dust. Tuberculosis was produced in 27 out of 29 tests.	Positive 27 of 29 tests	
Jones [64]	1900	The author inoculated guinea pigs interperitoneally with the mucus from the nostrils of 131 healthy persons. Three of these guinea pigs died of tuberculosis on the 8th, 14th and 59th days after inoculation. No tubercle bacilli could be found by stains of the nasal mucus[1].....		
Peterson [111]	1900	One of 8 guinea pigs became tuberculous after inhaling sputum, dried for 2 months; while sputum dried for 3 months gave one positive in 6.....	2 mo. 3 mo.	
Heymann [59]	1901	The aspirated air from sputum, dried 6 days and injected into guinea pigs, produced tuberculosis	6 days	

[1]It is probable that these three guinea pigs, or at least the first two, died of spontaneous tuberculosis.

TABLE 6—*Continued.*

Author	Date	Conditions	Not killed	killed
Heymann[59] (continued)		Dust from rooms of consumptives: Samples taken on a brush		
		No. of samples / No. of positive / Percent positive		
		Private rooms 59 / 5 / 8.5		
		Hospitals 61 / 5 / 8.2		
		Samples taken on a moist sponge		
		Private rooms 57 / 9 / 15.78		
		Hospitals 62 / 25 / 40.3		
		Total sa'ples 239 / 44 / 18.4		
		Hospitals 123 / 30 / 24.3		
		Pri'te homes 116 / 14 / 12.0		
Gotschlich[51]	1903	No tubercle bacilli were found in 119 samples of dust taken from 15 places where consumptives were in the habit of visiting.		
Hill[60]	1903	Samples of tuberculous sputum from 10 rooms, using 496 swabs, were taken by rubbing them on carpet, furniture, etc. In 8 of the 10 cases the sputum was proven to contain tubercle bacilli from stained preparations; the other two were not tested. Tubercle bacilli dried on glass rods were dead after 16 days.		
Noetel[105]	1904	Clothing that had been and that was being worn daily by consumptives was enclosed in a large box of three cubic meters content. The dust was beaten and shaken out and allowed to settle; then it was collected and thoroly rubbed up in 5 cc. of broth. The original material, and dilutions of .1 and .01 were respectively injected into guinea pigs:		
		Coat and vest worn daily	Positive	
		Jacket and hose worn daily:	Positive	
		New jacket, hose and old vest with no evidence of contaminated sputum.	Negative	
		Old coat	Positive	
		Coat, hose and plush vest, none worn for three weeks	Positive	
		Wool jacket and old hose, not worn for five weeks	Positive	
Cadeac[18]	1905	Tuberculous sputum exposed on glass to air and light	2, 4 and 6 dys.	4, 6 and 10 dys.
		Tuberculous sputum, in thick layers dried on a marble slab		14 days
Kirstein[69]	1905	Tubercle bacilli from pure culture or from tuberculous sputum were sprayed upon collected dust, and then after different lengths of time tested for virulence by inoculation of guinea pigs. Dust from:		

TABLE 6.—*Continued*

Author	Date	Conditions	Not killed	Killed
Kirstein[59] (continued)		Books and papers	8 days	14 days
		Sputum dust	4 days	7 days
		Cloth ravelings	5 days	10 days
		Street dust	3 days	8 days
Park and Williams[110]	1905	Dried tuberculous sputum gradually loses its virulence, but still infective	2 to 3 mo.	
Twitchell[149]	1905	Tuberculous sputum:		
		In paraffined bottles in dark moist box	170 days	188 days
		In paraffined bottles in diffuse light	124 days	175 days
		In paraffined bottles in thermostat	33 days	100 days
		In cotton-stoppered bottles in dark moist box	157 days	172 days
		In cotton-stoppered bottles in dark closet	100 days	141 days
		On ice	102 days	153 days
		Tuberculous sputum:		
		On handkerchiefs	70 days	110 days
		On towels	70 "	110 "
		On carpets	39 "	70 "
		In sand in moist light place	123 "	148 "
		In sand in dry light place	30 "	70 "
		In open bottles, out doors, winter	110 "	132 "
Sormani[138]	1906	Dried tuberculous excretion at 35° C.	15 days	
LeNoir and Camus[86]	1907	Large quantities of air were aspirated from tuberculous hospitals and the dust injected under the skin of guinea pigs. From 4 tests aspirating respectively 270, 2000, 20000 and 53000 liters, in no case were tubercle bacilli found		all
Rodet and Delanoe[120]	1907	Eleven samples of dust from local offices and private dwellings were inoculated into guinea pigs, with wholly negative results		11 samples
Köhlisch[71]	1908	Fresh tuberculous sputum or a pure culture of tubercle bacilli was intimately mixed with dust obtained from dwellings and factories, and the number of tubercle bacilli that a guinea pig inhales in a given time was determined. It was found that tuberculosis was produced in the test animals when as low as 50 tubercle bacilli were inhaled, and that the inhalation of 270 or more of these germs always produced tuberculosis. Another series of experiments were made with dust from 15 dwellings where consumptives were living. The rooms were small and poorly kept. Eighteen guinea pigs breathed for some time in dense clouds of this dust. None of these guinea pigs became tuberculous.		

TABLE 6.—*Continued*

Author	Date	Conditions	Not killed	Killed
Kuss[77]	1908	Tuberculous sputum dried in thin layers in the dark, free to the air:		
		Virulence wholly preserved	12 to 14 days	
		Virulence diminished	18 days	
		Virulence much decreased, yet a large dose produced a tuberculous guinea pig	20 to 30 days	
		Virulence entirely lost		40 to 50 days
		Tuberculous sputum, dried in thin layer, in diffuse light of a room rapidly decreased in virulence:		
		Noticeable decrease	3 days	
		Marked decrease	7 days	
		Very noticeable decrease	10 days	
		Not entirely lost	15 days	
		Entirely lost		20 days
		Two guinea pigs were exposed to the dust made by shaking of handkerchiefs on which tuberculous sputum had been dried for 25 days.		
		Neither became tuberculous		25 days
		Six guinea pigs were exposed to the dust from a plank containing sputum that had been drying for 17 days. Remained healthy		15 days
		Experiments in a box of 125-liter content:		
		Tuberculous sputum dried for 6 days in the dark was powdered and 1 to 2 grams suspended in the air of this box. Guinea pigs with their heads projecting into this box were made to breathe this dust from 30 to 60 minutes. In all cases the guinea pigs became tuberculous	6 days	
		Experiments in a room of 30 cubic meters:		
		Series 1.—125 cc. of tuberculous sputum were dried on a carpet from 11 to 13 days. Nine guinea pigs were swung 60 to 90 inches above this carpet. Then the carpet was beaten and swept for 20 minutes. Four of the 9 guinea pigs became tuberculous. Also the dust obtained by the aspiration of 60 liters of air, at a point 60 inches above this carpet, was inoculated into a guinea pig, which became severely tuberculous	11 to 13 days	
		Series 2.—20 cc. of tuberculous sputum were dried on another carpet for 6 days. Six guinea pigs were suspended above this carpet, which was beaten and swept for 12 minutes. Two of the 6 guinea pigs became tuberculous. Also		

TABLE 6.—*Continued*

Author	Date	Conditions	Not killed	Killed
Kuss[77] (continued)	1908	dust obtained by the aspiration of 71 liters of air produced a tuberculous guinea pig	6 days	
		Series 3.—Six of the 9 guinea pigs exposed to the beatings and sweepings of the two carpets of Series 1 and 2, described above, became tuberculous.....	6 to 13 days	
LeNoir and Camus[83]	1908	Tuberculous sputum rich in tubercle bacilli was mixed with dust a part of which had been sterilized, another part not sterilized, and each mixed sample was dried in a large open flask and kept in the laboratory for 33 days. Each sample was inoculated into a series of 5 guinea pigs:		
		Series 1.—Dust not sterilized. Three of the 5 guinea pigs became tuberculous only in the lymphatic glands near the point of inoculation[1].....	33 days	
		Series 2.—Dust sterilized. Three of the 5 guinea pigs died of acute infection; the other 2 had tuberculous lymph glands near the point of inoculation, and one of the two had a tubercle in one lung[1].....	33 days	
LeNoir and Camus[86]	1908	Again, the author examined dust from a tuberculous hospital:		
		Series 3.—The dust was collected from the cornice near the ceiling, wash-board, window-sill, window-seat, bed railings and floor. All was thoroly mixed, divided into two lots of .6 gram each, and placed in two flasks. One flask was protected from the light; the other was exposed to diffused light for 5 days and to sunlight 3 days. Each sample was inoculated into a series of 5 guinea pigs. In each case only one of the 5 test animals became tuberculous.		
		Series 4.—Four grams of dust were collected and treated as in Series 1. In this series 2 of the guinea pigs died of acute infection and 3 remained healthy.		
		Series 5.—Dust was collected and treated as in Series 1. 0.7 grams of the dust was placed in each of two flasks. The first was exposed		

[1]The tuberculosis may have been produced from dead tubercle bacilli.

TABLE 6.—*Continued*

Author	Date	Conditions	Not killed	Killed
		for 10 days to the sun; and the second in the shade for the same time. A series of 5 guinea pigs was inoculated from each sample. In each case 4 of the test animals died of acute infection within a few days; the other lived 17 days and then died of an unknown disease. Neither became tuberculous		10 days
LeNoir and Camus[85]	1908	The nasal cavities of non-tuberculous persons, living in a room with consumptives, were washed with wet cotton swabs and this material inoculated into guinea pigs. Of 9 guinea pigs thus inoculated 2 died with abscesses and the other 7 remained healthy.		
		Further investigations were carried out on consumptives. Of 13 guinea pigs inoculated 3 showed tuberculous changes.[1]		
Verdozzi[152]	1909	Dust from the library at Rome produced tuberculosis in rabbits.		
Rickards, Slack and Arms[119]	1909	Tuberculous sputum was placed on small strips of carpet and wooden tongue depressors and exposed in tenement houses in Boston:		
		To diffuse daylight	3 to 10 days	
		In the dark	10 to 14 days	
		To the sunlight	6 hrs.	
		The same exposed in the Boston Health Laboratory:		
		In diffuse daylight, on carpet	19 days	after 19 to 57 days
		In diffuse day light, on wood:		
		Positive	31 days	
		Negative		25, 29, 32 days and afterward
		In the dark exposed in the air on carpet:		
		Positive up to	35 days	
		Negative then till 65 days		36 to 65 days
		At this time (65 days) two specimens rubbed together were positive	65 days	
		On wood no end point was reached. Samples were positive up to	88 days	
LeNoir and Camus[82]	1909	Ten rabbits in one box and 4 in another were placed in a tuberculous hospital, and arranged so that they could breathe only dust-laden air from the rooms with tuberculosis patients. Four of the 10, and 2 of the 4 rabbits were found to be tuberculous when examined 6 weeks later.		

[1]Tuberculosis in these cases was more likely produced by fresh tubercle bacilli from the patient than from dust breathed by the patient.

TUBERCLE BACILLI IN WATER AND OTHER MATERIAL

INTRODUCTORY STATEMENT The fact that tubercle bacilli live and remain virulent in water for so long a time (one year and more), together with the other well-established fact of the danger of the ingestion of these germs, makes their presence in drinking water, in food, and in the soil assume special significance. Tubercle bacilli gain entrance to water supplies thru dejecta from tuberculous farm animals, especially dairy cattle, as well as from tuberculous people; in the latter case both from their dejecta and from their sputa. With these facts before us we can well conceive the danger of tuberculous infection from drinking water and the benefit that comes from a purified water supply.

MILLS-REINCKE PHENOMENON The epidemics of typhoid fever which at times have caused the death of hundreds of people in a city within a few weeks have frequently been traced to the water supply. This brought about the purification of the city water supplies. Besides the lessening of typhoid fever in a city having a purified water supply, there has been noted also great benefit from the lessening of other diseases. This was observed independently by J. J. Reincke, of Hamburg, Germany, and by Hiram F. Mills, of Lawrence, Massachusetts. Each was the health officer of his respective town. This fact was styled by Sedgwick and MacNutt[133] the Mills-Reincke phenomenon —"For every life saved from typhoid fever by the purification of a water supply, a certain number of lives are saved from other diseases."

The other diseases more especially concerned are infant diarrhea, tuberculosis, and bronchial troubles. Mr. Allen Hazen, at the International Engineering Congress held at the St. Louis Exposition in 1904, gave a mathematical equivalent to this phenomenon in what is now known as Hazen's theorem, viz., "Where one death from typhoid fever has been avoided by the use of better water, a certain number of deaths, probably two or three, from other causes have been avoided." Sedgwick and MacNutt found the Mills-Reincke phenomenon and Hazen's theorem to be sound and conservative when vital statistics of Lowell, Albany and Binghampton were carefully studied.

Dr. J. J. Reincke, health officer of Hamburg, in his report of 1893, has the following to say concerning the lessening of tuberculosis:

> "Especially surprising was the improvement (as to phthisis) in the period from the fourth to the sixth decade, when the sewage and the water-supply system just completed must have been of very strong influence for general cleanliness." (Page 285).

"It is worthy of notice that it is maintained from the surgical side that since the filtration of the water the number of cases of bone and joint tuberculosis has diminished extraordinarily. Further observations should tell whether a causal connection really exists here. At any rate such a possibility cannot be excluded, since of course the sputa and the bowel discharges of all the tuberculous reach the Elbe thru the sewers; and tubercle bacilli, just as well as typhoid bacilli, could have been carried thence in the water to the people." (Page 300).

Sedgwick and MacNutt find, by comparing the number of deaths from pulmonary tuberculosis for a period of five years just previous to water-supply purification with the five-year period just following, a decrease of 12 percent at Lawrence, Massachusetts, and a decrease of 14 percent at Lowell. Tho it is certain that this decrease is not wholly due to the better water supply, yet when compared with Manchester, Massachusetts, a city with a fair water supply, which supply had not been changed during this ten-year period, it is seen that the percentage of decrease in deaths from tuberculosis at Lowell and at Lawrence is much more marked than at Manchester. That drinking water has been and is still, in places, a source of tuberculous infection is probable.

IN FOOD AND SOIL

The frequency with which tubercle bacilli occur in market milk and butter is indicated by a tabular review of the literature upon this subject given in our previous publication (Briscoe and MacNeal, Bulletin 149 of this station). In 1233 samples of butter tubercle bacilli were found 163 times (13.2 percent); in 7397 samples of market milk they were found 502 times (6.8 percent). This frequency, together with the length of time that these germs are known to live in butter, indicates that this is an important source of infection for man, especially children. In soil and foul material they are known to live for a great length of time, and are there dangerous to farm animals.

A brief summary of the literature on the life of tubercle bacilli in water and in other material is given in Table 7.

TABLE 7.—DURATION OF LIFE OF TUBERCLE BACILLI IN WATER AND OTHER M

Author	Date	Conditions	Not killed	
Folk[41]	1883	In decaying matter an injury was observed after a few days	few days	
Schill and Fischer[129]	1884	In sputum, virulent	43 days	
Sormani[138]	1886	Tuberculous sputum remained virulent in water	12 mo.	
Chantemesse and Widal[22]	1888	Sterilized and unsterilized samples of water inoculated with pure cultures of tubercle bacilli were kept at:		
		A temperature of 8° to 12° C	50 days	
		Room temperature 15° to 20° C	70 days	
Galtier[46]	1888	Tuberculous spleen in water 3° to 8° C	17 days	
		Tuberculous products of pigs and cows in running and stagnant water at 17° to 0° C	14 days	
		Tuberculous products of a cow in running water 4° to 10° C	2 mo.	
Cadeac and Malet[17]	1888	Tuberculous lung, buried	77 to 167 days	after 176 days
		Piece of tuberculous lung in bowl of water exposed on outer window-sill	76 days	
		Same (repeated)	120 day	150 days
		Same (repeated) triturated with water, in air	16 days	67 days
Heim[55]	1889	In butter	30 days	
Straus and Dubarry[143]	1889	In river water (Ourcq):		
		at 20° C	27 days	
		at 35° C	35 days	
		at 38° C	95 days	
		In distilled water:		
		at 30° C	24 days	
		at 35° C	25 days	
		at 38° C	115 days	
Gärtner[44]	1890	In rotting flesh	7 mo.	
Freytag[43]	1890	Tuberculous sputum in an abundance of table salt for two weeks; then inoculated under the skin of a guinea pig caused generalized tuberculosis	2 wks.	
Schottelius[131]	1890	Buried phthisical lungs	2 yrs.	
Laser[79]	1891	In butter	4 wks.	
Petri[113]	1891	Organs containing tubercle bacilli:		
		Buried in a zinc box	3 mo. 6 days	
		Buried in a wooden box	1 mo. 5 days	
Stone[140]	1891	In putrefying sputum	3 mo.	
Ransome and Delepine[114]	1894	Very virulent sputum exposed to very little air in dark	16 days	
Löesner[88]	1896	Tuberculous organs placed in cadavers of hogs and buried	3 mo.	4 mo.
Schneiderlin[130]	1897	Tubercle bacilli remained 8 years in soil without losing their staining property	8 yrs.(?)	
Gaertner[48]	1898	In manure, temperature only 40° C,	3½ mo.	
Hance[52]	1898	Tuberculous sputum kept fluid in a well-stoppered bottle		17 mo.
Klein[70]	1899	Buried in dead mice and guinea pigs		7 wks.

TABLE 7.—*Continued*

Author	Date	Conditions	Not killed	Killed
[illegible][101]	1900	Tuberculous sputum mixed with river water:		
		In diffuse light, room temperature.	162 days	211 days
		In the dark virulence was greater..	162 days	211 days
		Sputum in canal water:		
		Diffuse light.....	131 days	197 days
		Kept in dark	131 days	197 days
		Exposed outdoors.....	131 days	197 days
		Sputum in sewage, room temperature	194 days	
		Sputa in sewage:		
		Exposed out doors	194 days	
		Kept in dark	105 days	
		Canal water mixed with sputa and garden soil kept at room temperature and received mid-day sun....	145 days	197 days
		Same as above except exposed to frost, snow and sunshine.....	148 days	
		Same as above except kept in sewage	150 days	
		Same as above except calcium chlorid added.	131 days	
Peterson[111]	1900	In sputum protected from the sun the bacilli almost unchanged morphologically.....	1½ mo.	
Petterson[112]	1903	In butter containing 4 percent salt ..		3½ wks.
		In butter containing 5 percent salt ..		4 days
		In salt flesh, not killed.....	4 wks.	
Dixon[32]	1908	Examined sewage from the tuberculous hospital of Philadelphia. Guinea pigs were inoculated, but previous to the inoculation the sewage was heated 60° to 70° C. from 2 to 15 minutes. No tuberculous guinea pigs were produced.[1].		
Schroeder[132]	1908	In butter as ordinarily salted.....	160 days	
Mohler, Washburn, Doan and Rogers[99]	1909	In butter in cold storage, pure culture or tuberculous udder.	6 mo.	
		Same in cheese.....	261 days	

[1]Temperature sufficient to pasteurize; most likely the organisms were killed by heating.

EXPERIMENTAL WORK

INTRODUCTION

The main purpose of this work was to determine the length of time that bovine tubercle bacilli live outside the animal body where they may be scattered by tuberculous farm animals, especially dairy cattle. As it was not feasible to keep tuberculous cattle at the Station, only a part of the work originally planned with naturally infected feces was done. In connection with this investigation some work has been done on tubercle bacilli of the human type and on other pathogenic and non-pathogenic organisms for purposes of comparison.

General Methods of Procedure

There are three points that need special attention: the sample, the manner of exposure, and the length of time required for life to become extinct in the culture exposed.

SAMPLE It is advisable that the sample of the organism undergoing test be a pure culture. In some cases where the organism is readily differentiated from contaminating growth, this is not so essential, as, for example, when pathogenic organisms are mixed with non-pathogenic organisms and the latter are readily eliminated by animal inoculation, or when a colored organism can be readily distinguished by its color characteristic. Even in these cases it is usually advisable to work with pure cultures.

EXPOSURE OF SAMPLE Much depends upon the manner of exposure; for instance, if exposure is to light it makes a decided difference whether the culture is exposed directly or in a glass container, or in the presence or in the absence of air. A complete statement as to the manner of exposure is absolutely essential to a definite interpretation of the reported results.

INDEX OF DEATH The most difficult point is to determine when life becomes extinct. This is comparatively simple with those organisms that readily grow on culture media, when they are exposed in pure culture. Their failure to grow gives a ready index of their death. With tubercle bacilli it it much more difficult to tell just when these organisms are dead. They cannot be cultivated readily from mixed growth since it is very difficult to prevent over-growth with contaminating organisms. Another difficulty is that dead tubercle bacilli produce typical tuberculosis in test animals (see page 312). It is therefore necessary to make animal inoculations in all cases where mixed tuberculosis materials are used, and from the original test animal, especially if only a local lesion is produced, a second healthy animal must be inoculated from some of the suspected tuberculous material. Suitable culture media should be seeded with this material from both the original and the secondary test animal. When all these tests prove characteristic for tubercle bacilli, it is evident that these germs are still living and virulent; when no infection, or only a local lesion, is produced in either the original or the secondary animal, and no growth occurs on the culture media, the tubercle bacilli are surely dead. In cases where the tuberculosis produced in the original inoculated animal from a small amount of material is severe and generalized, it may be con-

sidered that these germs are living, and this should then be verified by culturing on media suitable for the growth of tubercle bacilli.

LESIONS PRODUCED BY DEAD TUBERCLE BACILLI

PURPOSE OF THE EXPERIMENTS

The purpose of these experiments was to determine the difference in effect of dead tubercle bacilli and of living germs when injected into guinea pigs; also to find a means of determining when lesions were produced by living and when by dead germs. In order to determine this three experiments were made.

EXPERIMENT I

For the first experiment a pure culture of the bovine type of tubercle bacilli was used. A heavy emulsion of fresh culture was made in sterile distilled water. The emulsion showed a distinctly milky appearance. One cubic centimeter of this fresh emulsion was injected subcutaneously into Guinea Pig 993. The remaining portion of this emulsion was autoclaved at fifteen pounds pressure for ten minutes. Two guinea pigs, Nos. 994 and 995, were each injected subcutaneously with 1 cc. of the autoclaved emulsion. Guinea Pig 993 was killed 49 days later and showed generalized tuberculosis. Guinea Pigs 994 and 995, killed respectively 85 and 50 days after inoculation, were found to be healthy. Their weights continued to increase from the time they were inoculated until killed, and at no time did they show any physical signs of tuberculosis.

EXPERIMENT II

Before the conclusion of the first experiment a second was started in which an eight-day culture of the same strain of bovine tubercle bacilli was used. One milligram was removed and rubbed up in 4 cc. of broth. One loopful of this emulsion was diluted with 2 cc. of broth and injected interperitoneally into Guinea Pig 1026. The 4 cc. of broth were placed in streaming steam for one hour. Guinea Pigs 1027 and 1028 were each injected interperitoneally with 2 cc. of this steamed emulsion. Guinea Pig 1026 showed physical signs of tuberculosis ten days after injection. No physical signs of tuberculosis were observed in either of the two guinea pigs (1027 and 1028) that were inoculated with the killed culture. Thirty-five days after inoculation Guinea Pigs 1026 and 1027 were injected with 2 cc. of the tuberculin. Guinea Pig 1026 died from the effect of the tuberculin after eight hours; 1027 died after eighteen hours. Autopsy of 1026 showed severe generalized tuberculosis; that of 1027 showed a few lesions in the liver and a very slight enlargement of the inguinal lymphatics. Microscopic preparations stained for tubercle bacilli revealed none of these organ-

isms. Cultures on glycerine egg and the inoculation of a second guinea pig gave negative results. Guinea Pig 1028 was killed 41 days after inoculation, and the autopsy showed a small abcess at the point of inoculation and several lesions in the liver. All other organs were apparently healthy. A stained preparation revealed several tubercle bacilli a part of which showed signs of disintegration. Glycerine egg media and a guinea pig were inoculated with pus material from the point of inoculation and a liver abcess. Neither of these revealed living tubercle bacilli.

EXPERIMENT III A third experiment was made using the same strain of tubercle bacilli. An emulsion was made in sterile 0.8 percent salt solution. The emulsion showed a faintly milky appearance. Three tubes of glycerine egg were seeded with this emulsion of tubercle bacilli and Guinea Pig 1666 was injected interperitoneally with 1 cc. The remaining portion of the emulsion was divided into two parts: one was heated at 85° C. for ten minutes, the other was heated in the autoclave at 115° C. for ten minutes. With each of these portions three glycerine egg slants were seeded and a guinea pig was inoculated interperitoneally with 1 cc. The unheated portion gave excellent growth and produced severe generalized tuberculosis in Guinea Pig 1666. The part heated to 85° C. for ten minutes gave no growth on either of the three glycerine egg tubes after six weeks incubation at 38° C. Guinea Pig 1167, killed 67 days after inoculation, showed local tuberculosis in the right superior inguinal near the point of inoculation and a few small lesions in the liver. All other organs were apparently healthy. A stained preparation from pus material of these lesions showed tubercle bacilli. Cultures on glycerine egg and the inoculation of a second healthy guinea pig from this material gave negative results. The part heated to 115° C. for ten minutes neither gave growth on the glycerine egg media nor produced any effect when 1 cc. was injected interperitoneally into Guinea Pig 1168.

CONCLUSIONS

1. Dead cultures, when not killed at too high a temperature, produce tuberculous lesions in guinea pigs.

2. Secondary guinea pigs inoculated from tuberculous material from lesions produced by dead cultures always remain healthy.

3. In determining the length of time tubercle bacilli live when exposed to various conditions outside the animal body, it is necessary to inoculate a second healthy guinea pig, especially when only local lesions are produced in the guinea pig inoculated with the original material, in order to be sure the tubercles are not produced by dead tubercle bacilli.

EFFECT OF LIGHT UPON SPORE- AND NONSPORE-BEARING ORGANISMS

CULTURE

A few spore-bearing and nonspore-bearing organisms were tested by exposing them to the direct rays of the sun. The spore-bearing organisms, *B. subtilis* and *B. mesentericus vulgatus*, were grown upon peptoneless agar (made with three grams of beef extract and fifteen grams of agar per liter) for six days at 32° C. An examination of the cultures showed an abundance of well-developed spores. The vegetative cells of these two organisms were obtained by repeated growths in beef broth. The broth cultures were kept at 37° C. and repeated every twenty-four hours for six days. A microscopic examination at the end of this time showed no spores. *B. diphtheria* cultures were obtained from twenty-four hours' growth upon Loeffler's blood serum. All other organisms used were from fresh broth cultures grown at the optimum temperature for the organism tested.

MANNER OF EXPOSURE

The cultures grown on the solid media were suspended in 0.8 percent salt solution. A concentration of slightly milky appearing emulsion was formed. Then a loop of this emulsion was spread in a very thin smear on either a small slip of sterile glazed paper or a small sterile glass cover. These were then exposed to the direct rays of the sun for definite periods of time between the hours of ten in the morning and three in the afternoon. The exposed slips, with the exception of those containing *B. diphtheria*, were aseptically dropped into sterile broth and incubated at a temperature of 30° C. for one week. The exposed smears of *B. diphtheria* were seeded on Loeffler's blood serum and incubated at 37° C. for one week. The cultures were carefully examined for growth, and if growth was evident further tests were made to see if it was the same as the original culture or an accidental contamination.

RESULTS

A summary of the results is given in Table 8. It will be noted that the nonspore-bearing organisms were all killed in a few minutes. (½ to 6 minutes). While the spore-bearing organisms were not killed in the time exposed (180 minutes).

TABLE 8.—ORGANISMS EXPOSED TO DIRECT SUNLIGHT

Name of organism	Not killed	Killed
B. subtilis spores[1]	120 min.	
B. subtilis, spores[1]	180 min.	
B. mesentericus vulgatus, spores[1]	180 min.	
B. subtilis, vegetative cells	½ min.	1 min.
B. mesentericus, vegetative cells	1 min.	2 min.
B. prodigiosus		½ min.
B. diphtheria	6 min.	after 6 min.
B. coli	2 min.	3 min.
B. typhosus	1 min.	2 min.
B. violaceus	2 min.	3 min.

[1] Time of exposure was not continued longer; no end point was reached.

EFFECT OF DIRECT SUNLIGHT UPON TUBERCLE BACILLI

CULTURE AND EMULSION The effect of direct sunlight was tested several times upon cultures from three types of tubercle bacilli. The organisms used for exposure were always from active cultures grown upon glycerine egg from two to three weeks. A heavy emulsion was made by rubbing up some of the culture on the inside of the neck of a sterile glass-stoppered graduate flask with a sterile glass rod. From time to time a few drops of sterile 0.8 percent salt solution were added. At first the culture was rubbed into a fine paste with the addition of only a few drops of the salt solution; then about 6 cc. of 0.8 percent salt solution were added and the contents shaken thoroly. The emulsion then showed a decidedly milky appearance and was filtered thru sterile glass wool to remove the larger clumps. The examination of a stained preparation, made in a similar way as the smears that were used for exposure to sunlight, showed the individual organisms usually well separated. There were, however, clumps of twenty-five to thirty organisms still to be found.

PREPARATION AND EXPOSURE OF SMEARS Smears from the prepared emulsions were made upon small slips of sterile glazed paper. Pins pushed thru the corners of these small paper slips were then stuck into the bottom of a pasteboard box having a snug fitting lid. The box and the slips were sterilized at 150° C. for one hour. A small loopful of the emulsion was smeared in a thin layer upon each of the sterile glazed paper slips. These were exposed at once to the sun for the desired length of time. Triplicate smeared slips for each period of exposure were seeded upon culture media suitable for the growth of tubercle bacilli. The media used was 5 percent glycerine beef-juice agar or glycerine egg.

The glycerine egg was prepared by opening, aseptically, ten good fresh eggs and pouring the whole contents into a sterile flask. To the well-beaten eggs there were added 200 cc. of 5 percent glycerine beef broth. This emulsion was placed in sterile test tubes by means of a sterile Pasteur's bulb pipette. It was solidified, in a slanting position, at 73° C. for two hours. After placing the exposed slip on the surface of the media, it was rubbed and scraped by means of a small sterile platinum spatula so as to dislodge some of the dried tubercle bacilli, and a small amount of the glycerine egg or glycerine agar was smeared over this slip until it was thoroly moistened. The cotton stoppers were flamed, dipped into paraffine and pushed into the tubes, which were then stopped with paraffined stoppers. The cultures were incubated at 38° C. for one month and then carefully examined for growth.

RESULTS

Table 9 indicates the method of recording the results for the separate tests. A summary of the results of the various tests from the three types of cultures is given in Table 10. It is seen that these germs are killed in a very short time (1 to 4 minutes). These results agree with those of Weinzirl (see Table 2).

TABLE 9.—BACILLUS TUBERCULOSIS, HUMAN TYPE EXPOSED TO SUNSHINE
(Between 11 and 12 A. M., Nov. 2, 1911)

Time of exposure, minutes	Conditions	Result of growth	Remarks
0	Controls, not exposed.....	+	Excellent growth
0	" " "	+	" "
0	" " "	+	" "
½	Sun bright, no clouds.....	+	" "
½	" " " "	+	" "
½	" " " "	+?	Contamination
1	" " " "	+	Excellent growth
1	" " " "	+	Moderate growth
1	" " " "	+	" "
2	" " " "	—	No growth
2	" " " "	—	" "
2	" " " "	—	" "
4	" " " "	—	" "
4	" " " "	—	" "
4	" " " "	—	" "
6	" " " "	—	" "
6	" " " "	—	" "
6	" " " "	—	" "
10	Few clouds near the horizon	—?	Contamination mould
10	" " " " "	—	" "
10	" " " " "	—	" "

TABLE 10.—SUMMARY OF THE RESULTS FROM EXPOSING TUBERCLE BACILLI TO DIRECT SUNSHINE

Name of culture	Not killed, minutes	Killed, minutes
B. tuberculosis, human	1	2
B. tuberculosis, bovine	1	2
B. tuberculosis, avian	2	4

EFFECT OF DESICCATION UPON BACTERIA

PURPOSE OF WORK

The purposes of this work was to compare the effect of desiccation upon tubercle bacilli with that upon some nonacid-fast bacteria. Two types of tubercle bacilli were used, human and bovine. The nonacid-fast organisms used were two pathogenic organisms, *B. typhosus* and *B. diphtheria,* and of the nonpathogenic germs sporebearing and nonspore-bearing were used (see list in Table 11).

CULTURES AND EMULSIONS

The cultures were grown and the emulsions made in the same way as described in the section on the effect of light upon bacteria. Only fresh, active cultures were used. The tubercle bacilli were grown only upon glycerine egg.

MANNER OF EXPOSURE

The manner of preparing the organisms for exposure was also the same as that described in the last section, with the exception that the smears were made only upon sterile glazed paper slips. These smeared slips were exposed in a dark sheet-iron box that was well ventilated. Control slips of all the organisms tested were seeded a few minutes after the visible moisture had disappeared from the paper slips. Thereafter duplicate slips were seeded at the end of 12 hours and 1, 2, 4, 8, 12, and 16 days. The tubercle bacilli were cultivated upon glycerine egg at 38° C. for six weeks. In examining the cultures for growth of these germs it is necessary to scrape the surface and make stained preparations even when visible growth is not present, for in practically every case there will be one or two cultures between the evidently visible growth and the culture proven to contain no growth by the absence of organisms in stained preparations, in which the cultures will have only a few, usually invisible, colonies. This condition was not noted in the cultures from smears exposed to the sun.

The smears of *B. diphtheria* were cultivated upon Loeffler's blood serum at 37° C., and those of all other organisms were placed in broth and cultivated at the optimum temperature for each species.

RESULTS

The results are given in Table 11. These are the average results from two testings of all organisms used. Some other tests were made, but the results are only slightly different from the average of these two complete series. In each of these two series the organisms were all tested at the same time and under the same conditions, with the exception that the cultures were grown upon different media, as noted above. This may have made some difference in the results. The tubercle bacilli were grown upon glycerine egg media for approximately three weeks, while *B. violaceus,* for instance, was grown in broth for 24 hours at 30° C. The tubercle bacilli were emulsified in 0.8 percent salt solution and transferred to the sterile paper slips, while the *B. violaceus* were transferred directly from the broth to the slips. It may be that at least part of the difference in the length of time required to kill these organisms by desiccation is due to this difference of growth and age of the cultures.

It is seen that a very little longer time is required to kill the tubercle bacilli than other nonspore-bearing organism; and this slight difference may readily be produced by the protection of the glycerine clinging to these organisms from the culture media, or more likely the protection offered by the presence of clumps of tubercle bacilli. It is almost impossible to eliminate clumps from an emulsion of tubercle bacilli, while other organisms repeatedly grown in broth may be well separated.

The tubercle bacilli, therefore, cannot be put in the class of bacteria with spores, and they are very near to nonspore-bearing organisms, or the vegetative cells of spore-bearings organisms, as regards the effect of desiccation. They should be classed with the more resistant of the nonspore-bearers.

TABLE 11.—EFFECT OF DESICCATION UPON BACTERIA

Organism	Not killed, days	Killed, days
B. tuberculosis, human	4	8
B. tuberculosis, bovine	8	12
B. diphtheria	4	5
B. typhosus	3	5
B. subtilis, spores	35	
B. subtilis, vegetative cells	1	2
B. vulgatus, spores	35	
B. vulgatus, vegetative cells	2	3
B. coli	3	4
B. violaceus	1	2
B. prodigiosus	1	2
Sar. lutea	4	5
Sar. aurantiaca	4	5

DURATION OF LIFE OF BOVINE TUBERCLE BACILLI IN COW MANURE

PURPOSES OF THE EXPERIMENTS

The general purposes of these experiments was to determine how long bovine tubercle bacilli will live in cow manure when exposed to weather conditions in a pasture field. Two series of tests have been made concerning the resistance of these organisms when a pure culture is artificially mixed in cow manure exposed in the sunshine, and also when it is exposed in a place protected from the sunshine. In a similar way other tests have been made with naturally infected manure from a tuberculous cow.

Series 1

(Special Methods)

PREPARATION OF SAMPLES

Artificially Infected Manure.—The culture used in preparing this sample of artificially infected manure was a strain of bovine tubercle bacilli obtained from Dr. Theobald Smith of the Harvard Medical School. It was received and has since been kept upon glycerine agar. The strain readily produces an abundant growth and is quite virulent to test animals.

Four milligrams of the culture from two tubes of glycerine agar were removed and emulsified as described on page 315. The emulsion was diluted to 200 cc. with sterile salt solution. It was expected that the large amount of solution used would give a more uniform mixture of the tubercle bacilli in the cow manure.

The sample of manure was obtained from two cows of the dairy herd. It was taken by the method described in Bulletin 149 of this station; i. e., by injecting air into the rectum of the cow until stimulated to defecate. The feces were caught in a sterile pail and at once covered and brought to the laboratory. The 200 cc. emulsion of bovine tubercle bacilli was thoroly mixed with 1800 grams of the fresh cow manure. The infected manure was tested for virulence by inoculation of two guinea pigs. One gram was rubbed up in 50 cc. of 0.8 percent salt solution, 40 cc. of the emulsion was centrifuged and the sediment injected subcutaneously into two guinea pigs, both of which became infected with generalized tuberculosis.

Naturally Infected Manure.—The sample of naturally infected manure was obtained from a tuberculous cow (cow No. 56 from the dairy herd of this station) that had previously reacted to tuberculin. The sample was taken by the method described in our previous publication referred to above. Approximately three kilos

of fresh manure were obtained. The manure from this cow had been tested a number of times for tubercle bacilli by making subcutaneous inoculations of 1 cc. of a 2 percent emulsion of the fresh feces. Upon four occasions such tests gave negative results; three other tests, made respectively August 31, 1909, July 18, 1910, and August 16, 1910, produced tuberculous guinea pigs. The guinea pigs in none of the three tests became severely tuberculous; and two of the guinea pigs, one each in the last two tests, remained healthy. The last testing, August 16, 1910, was made on the day of exposing this sample of manure to the weather. Tho the infected guinea pig from this sample did not show severe tuberculosis when killed and examined 80 days after inoculation, a second guinea pig inoculated with the diseased tissue from this guinea pig showed severe generalized tuberculosis when killed and examined 38 days later.

EXPOSURE OF SAMPLES

The infected manures were taken to a secluded plot of ground on the Experiment Station farm for exposure. The manure infected with pure culture of bovine tubercle bacilli was divided into two equal parts. One half was placed in a free, open place, fully exposed to the sunshine thruout the whole day. This part, flattened out into a two-inch layer, was placed upon a sod with the grass cut short. A one-inch mesh poultry netting was placed over the infected manure in order, especially, to keep out the English sparrows, so that they could not carry upon their feet this infected manure to the stock upon the Experiment Station Farm.

The other part, protected from the sunshine, was placed a few yards from the former on the north side and very near a bank of earth six feet high. The ground upon which this manure was exposed was first made smooth by removing the sod. The manure was then spread in a two-inch layer just as was the part exposed in the sunshine. To protect this sample further from the light, an embankment of soil was made one foot high on three sides, and was covered over with a bunch of weeds. Tho the soil was more moist than in the plot where the part was exposed in the sunshine, it was not more moist than that usually found in a shady place. The layer of infected manure in this protected place dried on top in a week's time to a hard crust, but the bottom always remained moist. The manure from the tuberculous cow was divided into two parts; one part was placed in the sunshine, and the other in the place protected from the sunshine, very near the two artificially infected samples and exposed in the same way.

TESTING THE SAMPLES

The samples were tested almost exclusively by inoculation of guinea pigs following the method outlined in our previous publication. Stains of smears from the infected manure were made on a few occasions; but since it is impossible to distinguish between dead and living tubercle bacilli by examining stained preparations, no reliance is placed upon this test. The largest sample possible was used so as not to kill the guinea pigs by acute infection. The amount of sample varied, using the centrifuge sediment from 40 cc. of a 1½ to a 2½ percent emulsion of dried feces in 0.8 percent salt solution. The sample from the layers of infected manures was taken by cutting out pieces of the dried layer about one inch square in cross section and taking all the manure in this section down to the ground and a small layer of soil with the sample, so as to be sure to get any tubercle bacilli that might have passed into this top layer of soil just under the infected manure.

The testing of the sample was carried out with more than usual precautions, keeping in mind all the time the probability of producing tubercles in the guinea pig, with dead tubercle bacilli. To be sure that the tubercles in the infected guinea pigs were produced by living, virulent bacilli, the extent and rapidity of the disease was considered, and also the diseased tissues were tested for tubercle bacilli by staining smears, by cultures, and by inoculation of another guinea pig. If the second guinea pig became tuberculous, stains and cultures for tubercle bacilli from its diseased tissues were made. If, now, all these tests were characteristic for tubercle bacilli, it was considered reasonably certain that the tubercle bacilli found in these diseased tissues were alive and virulent. But when the guinea pig inoculated from the sample of manure became in a short time severely tuberculous, it was not thought necessary always to inoculate a second guinea pig from the diseased tissues of the former.

WEATHER CONDITIONS

The weather conditions during the time the manure infected with tubercle bacilli was exposed are given in Table 12.[1] The weather was not marked with any unusual occurrences. With the exception of the loss of the sunshine records from the third to the seventh of September, inclusive, when the electric sunshine recorder failed to work, the records are quite complete. This table records the date on which each test was made, the number of days of exposure to the time

[1] The data for this table were furnished us from the Laboratory of Soil Physics of the Department of Agronomy of this station by the kindness of Professor J. G. Mosier.

TABLE 12.—WEATHER CONDITIONS DURING THE EXPOSURE OF COW MANURE INFECTED WITH TUBERCLE BACILLI

(From the beginning, July 29, to time of test)

Date of test	Days exposed	Days			Hours of sunshine	Inches of rainfall	Temperature (degrees in Fahrenheit)				
							Daily				Average monthly
		Clear	Partly cloudy	Cloudy			Highest maximum since last test	Lowest minimum since last test	Average during time of exposure		
									Maximum	Minimum	
Aug. 5	7	2	5	0	57.50	.80	88	55	84	62	72.32
Aug. 15	16	3	13	1	117.75	1.02	88	58	84	62	72.32
Aug. 29	31	9	20	2	216.00	3.34	89	46	83	62	72.32
Sept. 16	49	13	33	3	348.25[1]	6.34	91	43	87	61	68.82
Oct. 18	81	30	55	6	593.00	7.01	87	43	83	59	64.54
Nov. 29	123	37	71	15	860.75	8.54	82	12	68	47	57.36
Jan. 16	171	43	98	30	919.38	11.13	53	4	61	41	51.00

[1]Records of five days in September were lost (from the 3d to the 7th inclusive.)

of the test; the number of clear days during this time and also the number of partly and of wholly cloudy days. There is also given the number of hours of sunshine, the amount of rainfall, the highest maximum daily temperature and the lowest minimum daily temperature occurring between each test; and an average daily maximum, and an average daily minimum temperature during the time of exposure up to the time the test was made. There is also given the average monthly temperature occurring during the time of exposure.

RESULTS OF THE TESTS

FROM ARTIFICIALLY INFECTED MANURE

Exposed in the Sunshine.—The results of testing the samples of cow manure infected with a pure culture of tubercle bacilli exposed in the sunshine are given in Table 13. As previously mentioned (page 320), the test of the sample on July 29, 1910, the day it was first exposed, produced two severely tuberculous guinea pigs. Since exposure seven tests have been made. On the 7th and 16th days the four guinea pigs inoculated contracted severe generalized tuberculosis. Only miscroscopic and cultural tests were made from the two guinea pigs inoculated on the 7th day of exposure, since the infection in both guinea pigs was so severe it appeared certain to be from living tubercle bacilli. This was later shown to be true from the culture tests. Microscopic and culture tests from the diseased tissues of both of the tuberculous guinea pigs infected with the sample taken on the 16th day of exposure, as well as an inoculation test from the tissues of one of them, showed characteristic tubercle bacilli. The test of the sample made 31 days since first exposed produced in both guinea pigs inoculated only slight tuberculosis of the right superior inguinal lymphatics. Microscopic, cultural, and inoculation tests of the diseased tissues from these guinea pigs showed typical tubercle bacilli. On the 49th day of exposure (September 16, 1910) the test showed that the virulence of the tubercle bacilli had considerably decreased, but was sufficient to produce slight tuberculosis in one of the two guinea pigs inoculated. The guinea pigs were inoculated subcutaneously with the centrifuge sediment of 40 cc. of an emulsion made by thoroly grinding three grams of the dried sample of infected manure in 150 cc. of 0.8 percent salt solution. The pus from the right superior inguinal of the guinea pig that became tuberculous was shown by miscroscopic, cultural, and inoculation tests to contain characteristic tubercle bacilli. The other guinea pig, when killed 53 days after inoculation, was found to be healthy. Tests made on

TABLE 13.—DURATION OF LIFE OF BOVINE TUBERCLE BACILLI IN COW MANURE EXPOSED IN SUNSHINE

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	757	7–29–10	46	Killed	Generalized tuberculosis, severe	+	+[2]	....	.....	.....	.....
0	758	7–29–10	46	Killed	Generalized tuberculosis, severe	+	—	.....	.....	.....	.....
7	763	8– 5–10	40	Killed	Generalized tuberculosis, severe	+	—	.....	.....	.....	.....
7	764	8– 5–10	40	Killed	Generalized tuberculosis, severe	+	—	.....	.....	.....	.....
16	771	8–15–10	45	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
16	772	8–15–10	45	Killed	Generalized tuberculosis, severe	+	+	820	Gen. tub.	+	+
31	780	8–29–10	67	Killed	Tub. of the R.S.[1] inguinal, slight	+	+	842	Gen. tub.	+	+
31	781	8–29–10	67	Killed	Tub. of the R. S. inguinal, slight	+	+	842	Gen. tub.	+	+
49	805	9–16–10	53	Killed	Tub. of the R.S. inguinal, slight	+	+	844	Gen. tub.	+	+
49	806	9–16–10	53	Killed	Healthy	.....	.....	.....	.....	.....	.....
81	824	10–18–10	49	Killed	Healthy	.....	.....	...	.....	.....	.....
81	825	10–18–10	49	Killed	Healthy	.....	.....	.....	.....	.....	.....
123	848	11–29–10	55	Killed	Healthy	.....	.....	.....	.....	.....	.....
123	849	11–29–10	55	Killed	Healthy	.....	.....	.....	.....	.....	.....
171	876	1–16–11	57	Killed	Healthy	.....	....	....	.....	.....	.....
171	877	1–16–11	39	Died	Acute infection, no tubercles	.....	.....	.....	.....	.....	.....

[1]Right superior.
[2]Cultured only on glycerine agar.

TABLE 14.—DURATION OF LIFE OF BOVINE TUBERCLE BACILLI IN COW MANURE EXPOSED IN THE SHADE

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	757	7–29–10	46	Killed	Generalized tuberculosis, severe	+	+[1]	.....	.....	.....	.....
0	758	7–29–10	46	Killed	Generalized tuberculosis, severe	+	−[1]	.....	.....	.....	.....
7	765	8– 5–10	40	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
7	766	8– 5–10	40	Killed	Generalized tuberculosis	+	+	.....	.....	.....	.....
16	773	8–15–10	45	Killed	Generalized tuberculosis	+	+	.....	.....	.....	.....
16	774	8–15–10	45	Killed	Generalized tuberculosis	+	+	.....	.....	.....	.....
31	782	8–29–10	68	Killed	Tub. of R.S. inguinal and lumbar lymphatic	+	+	843	Gen. tub., severe	+	+
31	783	8–29–10	68	Killed	Tub. of R.S. inguinal and slight tuberculosis in spleen	+	+	843	Gen. tub., severe	+	+
49	807	9–16–10	53	Killed	Generalized tuberculosis	+	+	845	Gen. tub., severe	+	+
49	808	9–16–10	53	Killed	Generalized tuberculosis	+	+				
81	828	10–18–10	5	Died	Acute infection	.....	.....	.....	.....	.....	.....
81	829	10–18–10	49	Killed	Healthy	.....	.....	.....	.....	.....	.....
123	850	11–29–10	55	Killed	Healthy	.....	.....	.....	.....	.....	.....
123	851	11–29–10	55	Killed	Healthy	.....	.....	.....	.....	.....	.....
171	878	1–16–11	54	Died	Acute infection, no tubercles	.....	.....	.....	.....	.....	.....
171	879	1–16–11	56	Died	Acute infection, no tubercles	.....	.....	.....	.....	.....	.....

[1] Cultured only on glycerine agar.

the 81st, 123d and 171st days of the exposure to sunshine showed the tubercle bacilli to be dead or at least not sufficiently virulent to produce tuberculosis when the centrifuge sediment was injected subcutaneously into guinea pigs.

It thus appears from these tests that pure cultures of bovine tubercle bacilli, when mixed with cow manure and exposed in an open place in a pasture field, remained alive in this instance for approximately two months.

Exposed in the Shade.—The part of the artificially infected manure exposed in a place protected from sunshine was tested on the same days and in the same manner as was the part exposed in the sunshine. These results are given in Table 14. The parts tested on the 7th, 16th, 31st and 49th days were shown at each testing to contain virulent tubercle bacilli. The infection of the guinea pigs in the first two tests was severe. The guinea pigs inoculated with samples taken on the 31st and 49th days of the exposure were infected with tuberculosis but not so severely as the guinea pigs inoculated on the 7th and 16th days. However, they were much more severely infected than the guinea pigs inoculated with the sample taken from the part exposed in the sunshine and tested upon the same days. The three samples taken on the 81st, 123d and 171st days were shown not to contain virulent tubercle bacilli. Three of the guinea pigs inoculated with the first two samples remained healthy until killed on the 49th and 55th days respectively after inoculation of the sample. One of the two guinea pigs inoculated with the sample taken on the 81st day died five days later with an acute infection. The two guinea pigs inoculated with the sample taken on the 171st day died of acute infection with no evidence of tuberculosis.

From these tests it appears that tubercle bacilli mixed with cow manure remain virulent to guinea pigs for 49 days after exposure in a place protected from the sun. All tests made later than this date showed these organisms to have lost their virulence. Virulence was retained longer in the shade than in the sunshine, as shown by the production of more severe tuberculosis in the guinea pigs inoculated from samples exposed in the shade than that produced in the guinea pigs inoculated on the same days with samples exposed in the sunshine.

FROM NATURALLY INFECTED MANURE

The tests of the samples of manure from the tuberculous cow, both the one exposed in the shade and the one exposed in the sunshine, at no time after exposure produced tuberculous guinea pigs. The results are tabulated in Tables 15 and 16. The sample exposed in the sunshine was tested on the 13th, 34th, 63d, and 105th days after first exposure. All the guinea pigs save one, which died of acute infection in four days, remained healthy until killed and examined 49 and 52 days after inoculation. The sample exposed in the place protected from sunlight was tested on the same days as the one exposed in sunshine, with one exception. No test was made on the 13th day of exposure from this part of the infected manure since there were not a sufficient number of guinea pigs available at that time. This sample was omitted because it was thought that the tubercle bacilli in the part protected from the sun would be the least likely to die. It was indeed unexpected that these bacilli would be dead in either of these two sampled at this time. It was a hot time in August. During the exposure from August 16 to 29 there was an average temperature of 72.32° F. and a rainfall of 2.32 inches, having six clear, one wholly cloudy, and seven partly cloudy days. A number of showers occurred, making it an excellent time for the growth of decay organisms found in the manure.

The killing of the tubercle bacilli in so short a time was no doubt due partly to the antagonism of the decay organisms and partly to the weakened virulence of these germs. Slight virulence was shown by the producing of only localized tuberculosis in one of the two control guinea pigs inoculated with a sample of the fresh manure. More experimental data upon this subject is very desirable.

TABLE 15.—DURATION OF LIFE OF TUBERCLE BACILLI IN MANURE OF COW 56, EXPOSED IN SUNSHINE

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	778	8–16–10	80	Killed	Tuberculous only in the R.S. inguinal and lumbar lymphatics	+	+	841	Gen. tub., severe	+	+
0	779	8–16–10	143	Killed	Healthy (10–26–10 two healthy young born)	.....	.....	....	.....	.....	.....
13	784	8–29–10	4	Died	Acute infection	.....	.....	.....	.....	.....	.....
13	785	8–29–10	68	Killed	Healthy	.....	.....	.....	.....	.....	.....
34	814	9–19–10	52	Killed	Healthy	.....	.....	.....	.....	.....	.....
34	815	9–19–10	52	Killed	Healthy	.....	.....	.....	.....	.....	.....
63	826	10–18–10	49	Killed	Healthy	.....	.....	.....	.....	.....	.....
63	827	10–18–10	49	Killed	Healthy	.....	.....	.....	.....	.....	.....
105	852	11–29–10	49	Killed	Healthy	.....	.....	.....	.....	.....	.....
105	853	11–29–10	49	Killed	Healthy	.....	.....	.....	.....	.....	.....

TABLE 16.—DURATION OF LIFE OF BOVINE TUBERCLE BACILLI IN MANURE OF COW 56, EXPOSED IN SHADE

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	778	8–16–10	80	Killed	Tuberculous only in the R.S. inguinal and lumbar lymphatics	+	+	841	Gen. tub., severe	+	+
0	779	8–16–10	143	Killed	Healthy (10–26–10 two healthy young born)	....	.....	....	.....	.....	.....
34	816	9–19–10	52	Killed	Healthy	.....	.....	....	.....	....	.....
34	817	9–19–10	52	Killed	Healthy	.....	....	.....	.....	.....	.....
63	830	10–18–10	56	Killed	Healthy	.....	....	.....	.....	.....	.....
63	831	10–18–10	56	Killed	Healthy	.....	....	.....	.....	.....	.....
105	854	11–29–10	55	Killed	Healthy	.....	.....	.....	.....	.....	.....
105	855	11–29–10	55	Killed	Healthy	.....	.....	.....	.....	.....	.....

Series 2

PREPARATION AND EXPOSURE OF SAMPLES A second series of experiments exposing cow manure artificially infected with a pure culture of bovine tubercle bacilli was made. The preparation and exposure of these samples were made in the same manner as in Series I (see pages 319 and 320). The places of exposure were within a few feet from the places where the samples were exposed in the first series.

WEATHER CONDITIONS Table 17 gives the weather conditions during the time of exposure of this series. The same plan is followed as in Table 12 giving the weather conditions in the first series. These conditions were just the reverse of those in the first series, the first series beginning in the hot month of August, the second in the cooler and more moist month of March. No especially unusual weather conditions occurred during this period.

RESULTS *Manure Exposed in the Sunshine.*—The results of exposing this infected manure in the sunshine are given in Table 18. The tubercle bacilli were still alive at the third test after 45 days of exposure. Tho the tuberculosis produced in each of the two guinea pigs from this test was only slight, as shown when these guinea pigs were killed and examined 53 days later, Guinea Pig 973, inoculated with the diseased tissues from these two, produced severe generalized tuberculosis. Typical cultures were obtained from the diseased tissues of both the original and the secondary inoculated guinea pigs. The samples taken after this date produced no tuberculosis in the six guinea pigs inoculated.

Manure Exposed in the Shade.—The results of exposing the sample in the shade are given in Table 19. Here we find that the tubercle bacilli were alive for 73 days, while in the sample exposed in the sun they were dead at this testing. Also the test made on the 45th day shows more severe tuberculosis in the guinea pigs inoculated from the sample kept in the shade than from the sample in the sun.

CONCLUSIONS 1. As shown by the results in both series of experiments, a pure culture of bovine tubercle bacilli mixed with cow manure and exposed in the sunshine in a pasture, remains alive and virulent for approximately two months.

2. The virulence of the tubercle bacilli in cow manure was retained in the samples protected from the sunshine longer than in those exposed in the sun, as shown both by the increased length

TABLE 17.—WEATHER CONDITIONS DURING THE EXPOSURE OF COW MANURE INFECTED WITH TUBERCLE BACILLI

Date of test	Days exposed	Days			Hours of sunshine	Amount of rainfall, inches	Temperature (degrees in Fahrenheit)				
							Daily				Average monthly
		Clear	Partly cloudy	Cloudy			Highest maximum since last test	Lowest minimum since last test	Average during time of exposure		
									Maximum	Minimum	
March 13	14	3	8	3	72	.95	69	11	44	26	33.66
Apr. 13	45	10	26	9	256	3.25	73	10	50	30	39.51
May 11	73	14	46	13	426	5.07	73	26	57	40	48.49
June 7	100	23	62	15	673	7.03	95	34	79	56	67.34
June 27	120	31	74	15	869	7.72	98	50	87	62	74.41
Aug. 11	166	47	94	15	1362	8.69	102	47	88	64	75.81

TABLE 18.—DURATION OF LIFE OF BOVINE TUBERCLE BACILLI IN COW MANURE EXPOSED IN SUNSHINE (Experiment 2)

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	913	2–27–11	9	Died	Acute infection	−	.....	.....	.....	.....	.....
0	914	2–27–11	51	Killed	Generalized tuberculosis, severe	+	+	....	.....	.....	.....
14	921	3–13–11	61	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
14	922	3–13–11	61	Killed	Generalized tuberculosis, severe	+	−	.....	.....	.....	.....
45	945	4–13–11	53	Killed	Tuberculous, slight	+	+	973	Gen. tub., severe	+	+
45	946	4–13–11	53	Killed	Tuberculous, slight	+	+	973	Gen. tub., severe	+	+
73	954	5–11–11	75	Killed	Healthy	.....	.....	.....	.....	.....	.....
73	955	5–11–11	91	Killed	Healthy	.....	.....	.....	.....	.....	.....
100	974	6–7–11	63	Killed	Healthy	.....	....	.....	.....	.....	.....
100	975	6–7–11	63	Killed	Healthy	.....	....	.....	.....	.....	.....
120	989	6–27–11	55	Killed	Healthy	.....	.....	.....	.....	.....	.....
166	1016	8–8–11	38	Killed	Healthy	.....	.....	....	.....	.....	.....

TABLE 19.—DURATION OF LIFE OF BOVINE TUBERCLE BACILLI IN COW MANURE EXPOSED IN THE SHADE
(Experiment 2)

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	913	2-27-11	9	Died	Acute infection	−	.....	.....	.....	.....	.....
0	914	2-27-11	51	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
14	923	3-13-11	51	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
14	924	3-13-11	67	Killed	Generalized tuberculosis, severe	+	−	.....	.....	.....	.....
45	943	4-13-11	37	Killed	Generalized tuberculosis	+	+	972	Tuberculous	+	+
45	944	4-13-11	37	Killed	Generalized tuberculosis	+	+	972	Tuberculous	+	+
73	956	5-11-11	75	Killed	Healthy	.....	....	....	.....	.....	.....
73	957	5-11-11	97	Killed	Tuberculosis, localized	+	+	1029	Gen. tub.	+	+
100	976	6- 7-11	63	Killed	Healthy	.....	.....	....	.....	.....	.....
100	977	6- 7-11	63	Killed	Healthy	.....	....	....	.....	.....	.....
120	990	6-27-11	71	Killed	Healthy	.....	....	....	.....	.....	.....
166	1017	8- 8-11	38	Killed	Healthy	.....	.. .	....	.....	.. .	.....

of time that they remained alive and by the severity of the disease produced in the guinea pigs inoculated upon the same days from each of these samples. This difference, however, was doubtless due mainly to the difference in drying.

3. Tubercle bacilli in the manure of a naturally infected cow were dead within two weeks after exposure. More experimental results are necessary before trustworthy conclusions can be drawn.

4. Danger of infecting farm animals from tuberculous manure is indicated.

DURATION OF LIFE OF BOVINE BACILLI IN GARDEN SOIL

PURPOSE OF TEST

Some investigators state that tubercle bacilli will live in soil and in dead carcasses buried in the soil from two to three years. If this be true, manure from tuberculous cattle when put upon cultivated fields which are later used to pasture stock may be a source of infection for farm animals, especially hogs. For this and other reasons it was thought advisable to test the length of time a pure culture of bovine tubercle bacilli will live in garden soil.

CULTURE

The culture of bovine tubercle bacilli was of the same strain as that used in the experiment for artifically infecting the cow manure. About four milligrams of pure culture were obtained from a four-weeks' growth on the surface of two large tubes of glycerine agar. The organisms were carefully removed from the glycerine agar and emulsified as described under "Cultures and Emulsions," page 315.

SAMPLE OF SOIL

The sample of soil with which the tubercle bacilli emulsion was mixed was obtained from a garden plot that had been in cultivation only two years. Previous to that time, this plot of ground had been in sod for at least fifteen years. The part from which the soil sample was obtained had been well manured with horse manure the first year it was under cultivation. The second year no manure was added but the ground was well stirred and made into a lettuce bed. A sufficient amount of this soil was obtained to fill a ¼-inch mesh wire basket having the dimensions of 4x5x6 inches. This required 1700 grams. This pulverized soil was placed in a large pan and the emulsion of tubercle bacilli sprinkled over it. These were thoroly mixed by being constantly stirred for some time. The amount of emulsion was sufficient to make the soil quite wet and sticky.

PLACE OF EXPOSURE

Ten grams of this infected soil were removed to be tested for tubercle bacilli as a control. The remaining soil was put into the wire basket and covered with a wire gauze. It was buried in another garden that had been under cultivation for one year and had received no manure or fertilizer of any kind. The place where it was buried had been previously well pulverized. The basket with the infected soil was buried six inches under the surface of the ground.

TESTS OF SAMPLES

Samples of this infected soil were tested for tubercle bacilli on the first day of exposure and on the 7th, 16th, 34th, 55th, and thereafter about once a month for 352 days. Ten grams of soil were removed by digging down beside the mouth of the basket and with a sterile potato knife making an opening to the center of the basket. After the removal of the sample, the opening was filled by pressing the soil in around this opening with a potato knife. The wire gauze was placed over the mouth of the basket and the garden earth filled in over it to a depth of six inches. The soil sample taken to the laboratory was thoroly shaken with 200 cc. of 0.8 percent salt solution in a 300 cc. flask. After standing ten minutes until the coarser sediment had fallen to the bottom, 40 cc. were removed and placed in two sterile centrifuge tubes and centrifuged for five minutes. The supernatant liquid was drawn off and put into two other centrifuge tubes and centrifuged for thirty minutes at 2000 revolutions per minute. The supernatant liquid was drawn off and discarded. The last 5 cc. of the liquor and sediment were thoroly mixed and injected subcutaneously into guinea pigs, in graded doses, giving one 1½ cc., another 1 cc., and for the first test a third received ½ cc.

RESULTS OF THE TEST

The results considering the length of time that tubercle bacilli live in the soil are recorded in Table 20. The guinea pigs inoculated from samples taken on the day of exposure, and on the 7th, 16th and 34th day after exposure showed in each case, when killed and examined, severe generalized tuberculosis. Microscopical and cultural tests showed the germs from the diseased tissues to be characteristic of active, living tubercle bacilli. Tests after this time indicated a weakening in virulence, but slight tuberculosis was produced in the test animals from material taken on the 213th day of exposure. Microscopic, cultural, and guinea-pig tests from the diseased tissues of the original guinea pig showed the tubercle bacilli in the soil sample to be active and virulent. Five tests were made after this date. In every case the guinea pigs, when killed and examined, were found to be healthy. The two testings after the last one in which tubercle bacilli were found, viz., the ones made on the

TABLE 20.—VIABILITY OF BOVINE TUBERCLE BACILLI IN GARDEN SOIL.

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	754	7–26–10	36	Killed	Tub. only in R.S. inguinal, slight	+	+	.....	.....	.....	.....
0	755	7–26–10	36	Killed	Tub. only in R.S. inguinal, slight	+	+	....	.....	.....	.....
0	756	7–26–10	49	Killed	Generalized tuberculosis, severe	+	+	803	Gen. tub., severe	+	+
7	761	8– 2–10	42	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
7	762	8– 2–10	42	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
16	767	8–11–10	46	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
16	768	8–11–10	46	Killed	Generalized tuberculosis, moderate	+	+	.....	.....	.....	.....
3	786	8–29–10	72	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
3	787	8–29–10	72	Killed	Generalized tuberculosis, severe	+	+	....	.....	.....	.....
5	818	9–19–10	53	Killed	Tub. at point of inoculation and in R.S. inguinal	+	+	846	Gen. tub., severe	+	+
5	819	9–19–10	53	Killed	Generalized tuberculosis, not severe	+	+	846	Gen. tub., severe	+	+
86	832	10–20–10	39	Killed	Generalized tuberculosis	+	+	847	Gen. tub., severe	+	+
86	833	10–20–10	39	Killed	Tuberculosis only at the point of inoculation	+	....	....	.....	.....	.....

TABLE 20—*Continued.*

137	863	12–10–10	2	Died	Acute infection	....	....	....	.....	.....	.....
137	864	12–10–10	42	Died	Generalized tuberculosis	+	...	....	.....	.....	.....
158	874	12–31–10	52	Killed	Slightly tuberculous	+	+	904	Acute infection	.. ..	.....
158	875	12–31–10	5	Died	Acute infection	.....	.....	....	.....	.....	.....
191	887	2– 2–11	2	Died	Acute infection	.....	....	....	.....	.....	.....
191	888	2– 2–11	25	Died	Acute infection	+	.....	919	Gen. tub., severe	+	+
213	905	2–24–11	3	Died	Acute infection	.....	.....	.....	.....	.....	.....
213	906	2–24–11	51	Killed	Tub. in R.S. inguinal and spleen	+	+	952	Gen. tub., severe	+	+
230	925	3–13–11	67	Killed	Healthy[1]	.....	.....	....	.....	.....	.....
230	926	3–13–11	67	Killed	Healthy[1]	.....	....	....	.....	.... ..	.....
261	947	4–13–11	53	Killed	Healthy[1]	.....	....	....	.....	.....	.. ..
261	948	4–13–11	53	Killed	Healthy[1]	.....	.....	....	.....	.. ..	.....
290	965	5–12–11	75	Killed	Healthy	.....	....	.....	.....	.....	.....
290	966	5–12–11	75	Killed	Healthy	.....	....	.....	.....	.....	.....
318	979	6– 9–11	61	Killed	Healthy	.....	.....	.....	.....	.....	.....
318	980	6– 9–11	88	Killed	Healthy	.....	....	....	.....	.....	.....
352	1002	7–13–11	43	Killed	Healthy	.....	.....	.....	.....	.....	.....

[1]The right superior inguinal enlarged in the third and fourth week and then became normal again.

230th, and on the 261st days of the exposure, produced in the third and fourth weeks an enlargement in the right superior inguinals of each guinea pig inoculated, which then became normal again. Tho it cannot be surely known, it may be that the number of live tubercle bacilli was so small as not to cause the disease, but of sufficient number to produce an enlargement of the glands. Three tests made later than these two showed no such enlargement of these glands.

BOVINE TUBERCULOSIS IN A DEAD ANIMAL

SOURCE OF MATERIAL

Besides the exposure of the pure culture of bovine tubercle bacilli in garden soil, a guinea pig that had died of bovine tuberculosis was exposed in this same soil. This guinea pig was extensively tuberculous. It was placed in a flower pot and covered with a screen of wire having a mesh fine enough to exclude earthworms. The screen was pressed down close over the guinea pig, which allowed the fine garden earth to come into immediate contract with the dead body. The abdominal and thoracic cavities had previously been opened in making an examination of the guinea pig just before placing it in the garden soil.

METHOD OF TESTING

At the time of the first test, made the 71st day of the exposure, the tissues of the guinea pig were so decayed that the flesh and the skin were easily torn. A part of the tuberculous lung and of the right superior inguinal lymphatic was removed for the test. At the next test, on the 99th day, most of the soft tissues had been carried away by small ants. On the 133d day only the bones, hair and some tendons remained. A few pieces of bones and a bunch of hair were obtained for this test.

RESULTS

The results of these tests are given in Table 21. Live, active tubercle bacilli were found on the 71st day; after this date no tuberculosis was produced in any of the guinea pigs inoculated. No doubt if the small ants had not molested the soft tissues, tuberculosis would have been produced in the test animals at later dates. While no final conclusion can be drawn, it is evident that these germs live a sufficient time in dead tuberculosis animals to be dangerous to stock.

TABLE 21.—VIABILITY OF BOVINE TUBERCLE BACILLI FROM A TUBERCULOUS GUINEA PIG BURIED IN GARDEN SOIL

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	914	3–2–11	51	Killed	Generalized tuberculosis, severe	+	+	....	.....	.....	.....
71	963	5–12–11	85	Died	Generalized tuberculosis, severe	+	.....	.....	.....	.....	.....
71	964	5–12–11	74	Killed	Generalized tuberculosis, severe	+	+	1007	Gen. tub., severe	+	+
99	981	6–9–11	61	Killed	Healthy	.....	.....	....	.....	.....	.....
99	982	6–9–11	61	Killed	Healthy	.....	.....	....	.....	.....	.....
133	1003	7–13–11	43	Killed	Healthy	.....	.....	.....	.....	.....	.....
162	1024	8–11–11	38	Killed	Healthy	.....	.....	.....	.....	.....	.....
162	1025	8–11–11	2	Died	Acute infection	.....	.....	.....	.....	.....	.....

DURATION OF LIFE OF TUBERCLE BACILLI IN WATER

PURPOSE AND PLAN OF TESTS The purpose of these tests was primarily to determine how long bovine tubercle bacilli will live in a watering tank such as is found on the average stock farm in Illinois. In connection with this work it was desired to test the length of time human tubercle bacilli will live in drinking water. With this in view experiments were planned and carried out with tubercle bacilli from the following sources:

1. Pure cultures, Series 1.
2. Pure cultures, Series 2.
3. The diseased tissues of a tuberculous guinea pig.
4. Tuberculous sputum.

Series 1. Pure Cultures

CULTURES The bovine culture was the same as that used in the cow manure which was exposed to weather conditions. The human culture was taken from the tuberculous glands of the neck of a patient from the Burnham hospital and isolated in this laboratory. The tissues were received May 25, 1910. A pure culture was obtained by inoculating a guinea pig and culturing the diseased tissues of the guinea pig. This culture, which is typical for the human type, has since been kept upon glycerine agar.

SAMPLES OF WATER The sample of water used to make the emulsion for exposing the bovine tubercle bacilli was obtained from the large watering tank used to water the dairy cattle of this station. This tank is 2½x4x10 feet, and is supplied with running water. A considerable amount of spirogyra and various kinds of smaller green algae was floating in the tank. Diatoms were also abundant. A green scum covered a large portion of the surface of the water. The sample was obtained in a 500 cc., sterile, cotton-stoppered flask. The water in the tank was slightly agitated with a stick about as much as it was thought the cattle would stir the water by drinking. The mouth of the flask was plunged under about six inches and then allowed to fill. The sample was at once taken to the laboratory and the emulsion prepared that same afternoon. The spirogyra died soon after the emulsion was made, but the diatoms and the smaller green algae, as well as numerous bacteria, were present at the conclusion of the experiment December 5, 1911, 586 days after the experiment started. The sample of water used to make the emulsion with the pure culture of human tubercle bacilli was taken from the tap water of the bacteriological laboratory. There were at the

time some algae and bacteria present in this water, a part of which were still living at the last test made.

PREPARATION OF EMULSIONS The emulsions both of the bovine and of the human cultures of tubercle bacilli were prepared in the same way. About two milligrams of a four weeks' culture grown upon glycerine agar were emulsified in the manner described under "Cultures and Emulsions," page 315. The emulsion of bovine tubercle bacilli was diluted to 350 cc. with the tank water obtained for this purpose; 250 cc. were used to expose in the running water, and 100 cc. to expose in standing water. In a similar way a 250 cc. emulsion of human tubercle bacilli was made.

EXPOSURE OF SAMPLE For exposing the emulsions in running water two 6-inch flower pots were obtained. The small opening in the bottom was stopped with a cork and this was sealed over with boiler paint. A test of the two flower pots thus prepared showed that they held water. After immersing them for twenty-four hours to within two inches of the top, no water rose on the inside, tho it became very moist. The two emulsions were now poured into these two flower pots and

FIG. 1. THE VESSELS AND THE POOL OF RUNNING WATER IN WHICH THE TUBERCLE BACILLI WERE EXPOSED.

The intake is at A; the outlet at B. The submerged vase C has been placed upon the shelf with other vessels so it can be seen. The flower pot D, which contained the tuberculous guinea pig, is placed upon a brick to bring it into view. The two unglazed cylinders and one of the flower pots show the effect of freezing.

each flower pot placed in a gallon glass jar containing water and immersed about three inches, so that the level of the emulsion of tubercle bacilli on the inside was the same as the level of the water on the outside. The water in the glass jars around the outside of the flower pots was kept continually running. While this procedure does not give the same condition as water running directly into the emulsion of bacteria, it allows to some extent the circulation of the water inside the porous flower pot with running water on the outside of these vessels. Another part of the emulsion of bovine tubercle bacilli was kept in a cotton-stoppered glass bottle. This bottle was partly immersed in one of the glass jars of running water. The water could in no way circulate in this bottle, but it was kept at approximately the same temperature as the emulsions in the flower pots.

The samples were kept in the laboratory until April 15, 1911, 250 days after the emulsions were first made. It was then found neither convenient nor desirable to keep them there longer. A pool was prepared in the courtyard of the Agricultural Building, to which they were transferred. The pool was made by sinking a large tile three feet in diameter and filling in the bottom with concrete. Constantly running water was maintained to a depth of twenty inches the year round. It did not freeze any time during the winter of 1911-12. A shelf of slate was placed four inches under the surface of the water on which the vessels containing the tubercle bacilli were set. The pool was screened against flies and other insects. (See Figs. 1 and 2.)

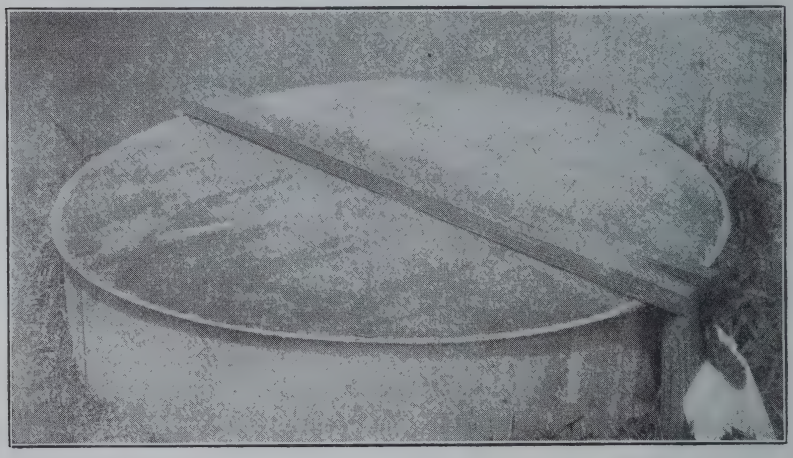

FIG. 2. THE POOL SCREENED AGAINST FLIES.

TESTING THE SAMPLES

Nineteen tests from these samples were made for the presence and for the virulence of tubercle bacilli. These tests were made on the 9th, 23d, and 44th days, and thereafter about once a month until the last test, which was made December 5, 1911, 586 days from the beginning. The sample of the bovine tubercle bacilli kept in the cotton-stoppered bottle was tested for only 202 days, since the sample was exhausted at this time. This sample was not transferred to the pool.

Each of the three samples was tested upon the same days and in the same manner. The water in the two flower pots was thoroly agitated by giving it a circular motion. With a sterile glass rod the sides and bottom of the flower pot were scraped so as to loosen adhering sediment that might contain tubercle bacilli. The water in the bottle containing the bovine tubercle bacilli was thoroly shaken before the test sample was taken. It was not convenient nor thought necessary to rub the inside of this bottle, as was done in the case of the flower pots. With a sterile Pasteur bulb pipette from each of the three containers approximately 5 cc. of this infected water were removed and placed in a sterile centrifuge tube and centrifuged for thirty minutes at a high speed. Four cc. of the supernatant liquid were removed and discarded. One or two drops of the sediment were placed upon a glass slide and a microscopic preparation made and stained for tubercle bacilli. The remaining sediment and liquid in the centrifuge tubes, about 1 cc. in quantity, was thoroly mixed and injected subcutaneously into a guinea pig. The same precautions were taken with these tests as in the case of the tuberculous manure to guard against mistaking tubercles produced by dead tubercle bacilli for those produced by living, virulent ones. Microscopic preparations and cultures were always prepared to test the diseased tissues of the infected guinea pig for tubercle bacilli. In a part of the cases, however, the tuberculosis was so extensive that it was not thought necessary to make inoculations of diseased tissue into another guinea pig.

RESULTS OF THE TESTS

The results of the tests to determine the length of time tubercle bacilli live in water are recorded in Tables 22, 23 and 24. With the first four tests of each of the three samples containing tubercle bacilli the guinea pigs became extensively and severely tuberculous, as will be shown by an examination of the three tables. Further tests of the diseased tissue from all these guinea pigs showed the tubercle bacilli to be living and virulent. The fifth test, made after 126 days, showed at least an apparent weakening of the virulence of these germs.

TABLE 22.—DURATION OF LIFE OF HUMAN TUBERCLE BACILLI IN RUNNING WATER

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
9	777	8–15–10	29	Killed	Generalized tuberculosis, severe	+	+	804	Gen. tub., severe	+	+
23	790	8–29–10	51	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
44	811	9–19–10	52	Killed	Generalized tuberculosis, severe	+	+	.....	....	.....	.....
75	836	10–20–10	53	Killed	Generalized tuberculosis, severe	+	—	865	Gen. tub.	+	+
126	861	12–10–10	45	Died	Generalized tuberculosis, severe	+	[1]	.....	.. ..	....	.....
126	862	12–10–10	72	Killed	Generalized tuberculosis, severe	+	+	902	Acute infection	... ...	.....
147	872	12–31–10	52	Killed	Generalized tuberculosis, severe	+	+	903	Acute infection	.....	.....
147	873	12–31–10	4	Died	Acute infection	.....	....	.....	.....	.....	....
180	893	2– 2–11	22	Died	Tuberculosis point of inoculation	+	+	916	Died acute infection	.. .	.....
180	894	2– 2–11	24	Died	Tuberculosis point of inoculation	+	.....	....	.....	... .	.....

TABLE 22—*Continued.*

202	911	2–24–11	12	Died	Acute infection	....	.....	..	.....	.....	.....
202	912	2–24–11	19	Died	Acute infection	... .	.....	.. ..	.....	.....	.....
221	930	3–15–11	63	Killed	Generalized tuberculosis, severe	+	+[2]	969	Gen. tub.	+	.....
221	931	3–15–11	63	Killed	Generalized tuberculosis, severe	+	—[2]	.. ..	.....	.....	.....
248	942	4–13–11	37	Killed	Tuberculosis	+	+	971	Gen. tub., severe	+	+
278	961	5–11–11	75	Killed	Generalized tuberculosis, severe	+	+	1009	Gen. tub., severe	+	+
307	985	6– 9–11	50	Killed	Generalized tuberculosis, severe	+	+	.....	Not made	.....	.....
441	997	7–13–11	40	Killed	Tub. of the R.S. inguinal	+	+	1036	Gen. tub., severe	+	+
470	1020	8–11–11	18	Died	Acute infection	... ...	.. .	.....	.....	....	.....
501	1055	9–11–11	54	Killed	Healthy	... .	.....	.....	.....	.....	.....
537	1072	10–17–11	42	Killed	Healthy	.....	. ..	.. ..	....	....	.....
565	1094	11–14–11	40	Died	Acute infection not tuberculous	... .	.....	..	.....	.....	.....
586	1106	12– 5–11	48	Killed	Healthy	....	...	.....	....	.....	.....

[1]Found dead, partly decayed, no cultures were attempted.
[2]A stain from each of four glycerine egg tubes seeded with pus from a lymphatic gland showed no growth, apparently all dead.

TABLE 23.—DURATION OF LIFE OF BOVINE TUBERCLE BACILLI IN A FLOWER POT PLACED IN RUNNING WATER

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
9	775	8–15–10	46	Killed	Generalized tuberculosis, severe	+	+	822	Gen. tub., severe	+	+
23	788	8–29–10	49	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
44	809	9–19–10	51	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
75	834	10–20–10	53	Killed	Generalized tuberculosis, severe	+	+	866	.....	.....	.....
126	857	12–10–10	32	Killed	Generalized tuberculosis, slight	+	.....	884	Gen. tub., severe	+	+
126	858	12–10–10	32	Killed	Generalized tuberculosis, slight	+	.....				
147	868	12–31–10	57	Killed	Generalized tuberculosis, severe	+	.....	.....	.....	.....	.....
147	869	12–31–10	50	Died	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
180	889	2–2–11	34	Killed	Tub. of R. S. inguinal only	+	+	927	Gen. tub., severe	+	+
180	890	2–2–11	25	Died	Tub. at point of inoculation only	+	1	920	Tuberculous	+	cont.

TABLE 23.—*Continued.*

202	907	2–24–11	13	Died	Acute infection, no tubercles	.....	.....	....	.....	.. .. .	.....
202	908	2–24–11	14	Died	Acute infection, no tubercles	.....	....	....	. .....	. . ...	.....
221	928	3–15–11	66	Killed	Generalized tuberculosis, severe	+	+		.....	.....	.....
222	929	3–15–11	63	Killed	Generalized tuberculosis, severe	+	+	968	Gen. tub., severe	+	.....
248	941	4–13–11	34	Killed	Generalized tuberculosis	+	+	....	.....	... .	.....
278	960	5–11–11	75	Killed	Generalized tuberculosis, severe	+	cont.	.....	....	.....	.....
307	986	6–9–11	50	Killed	Generalized tuberculosis, severe	+	+	...	.....	.....	.....
441	998	7–13–11	40	Killed	Generalized tuberculosis, severe	+	+	1033	Gen. tub.	+	+
470	1021	8–11–11	38	Killed	Healthy	....	....	....	.....	.....	.....
489	1046	8–30–11	69	Killed	Healthy	.....	...	.....	.. ..	.. ....	.....
501	1056	9–11–11	54	Killed	Healthy	... ...	.	...	.....	.. .	.....
537	1073	10–17–11	42	Killed	Healthy	. .....	.....	....	... .	.....	....
565	1095	11–14–11	44	Died	Acute infection, no tubercles	.....	....	....	.....	.....	....
586	1107	12–5–11	48	Killed	Healthy	.....	.....	.....	.....	.. ..	.....

[1] Not enough material for cultures.

TABLE 24.—DURATION OF LIFE OF BOVINE TUBERCLE BACILLI IN STANDING WATER

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
9	776	8–15–10	46	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.... ..	.....
23	789	8–29–10	49	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.. ....	....
44	810	9–19–10	52	Killed	Generalized tuberculosis, severe	+	+	....	.....	.... ..	.....
75	835	10–20–10	53	Killed	Generalized tuberculosis, severe	+	+	867	.....	.. ....	.....
126	859	12–10–10	32	Killed	Tuberculosis, slight	+	+	859	.....	... ..	.....
126	860	12–10–10	32	Killed	Tuberculosis, slight	+	+	...	.....	.. ....	.....
147	870	12–31–10	51	Killed	Generalized tuberculosis, severe	+	+	....	.....	.....	....
147	871	12–31–10	51	Killed	Generalized tuberculosis, severe	+	+	901	Gen. tub. severe	+	+
180	891	2–2–11	24	Died	Acute infection, no tubercles	.....	...	....	.....	... ..	.....
180	892	2–2–11	24	Died	Acute infection, no tubercles	.....	....	.....	.....	.....	.....
202[1]	909	2–24–11	78	Killed	Doubtful	.. .	....	967	Gen. tub.	+	+
202	909	2–24–11	7	Died	Acute infection	....			....	....	

[1] This sample used all the water from the bottle.

The tuberculosis produced in the two guinea pigs inoculated at the fifth testing from the sample of standing water contained in the cotton-stoppered bottle, was neither extensive nor severe. Those from the running water had a higher virulence than the tubercle bacilli in the standing water, but the tuberculosis produced was not so severe as that from the former samples. It should be noted that the time of killing these guinea pigs was much earlier, and it may be for this reason alone that the virulence appeared to be weakening. This fact was noted at the time of killing the four guinea pigs inoculated with the bovine tubercle-bacilli-infected waters, and so the two guinea pigs inoculated with water infected with the tubercle bacilli of the human type were not killed at this time. Thirteen days later one of these two guinea pigs died with generalized tuberculosis. Unfortunately the death of this guinea pig was not noted until a short time afterwards, and it could not be determined whether tuberculosis was the only cause of death. The other of the two guinea pigs inoculated with the sample of water containing the human type was killed 72 days later, and the examination showed severe generalized tuberculosis.

Later, on the 147th day, we find severe generalized tuberculosis produced from each of the three samples. After this date only two more tests were made from the sample in the cotton-stoppered bottle, one on the 180th and the last one on the 202d day of exposure. These two tests show the lessening of the virulence of the germs: for from the former no tubercles could be noted 24 days later, when the two inoculated guinea pigs died of acute infection; from the latter, one guinea pig died too early to make the test; the other, killed 78 days after inoculation, showed only a doubtful sign of tuberculosis. However, an inoculation of an emulsion of the tissues from the point of inoculation and the right superior inguinal glands produced, in a secondary guinea pig, generalized tuberculosis. From the diseased tissues of this guinea pig an active culture was obtained. Thus no end point was reached from the sample of standing water with the bovine tubercle bacilli in the cotton-stoppered bottle. The other two samples, kept in porous flower pots which were transferred to the pool in the courtyard, remained alive for 441 days, but were dead on the 470th day.

From the emulsion of the bovine type severe tuberculosis was produced in the guinea pigs even in the last test in which these germs were found alive. From the emulsion of the human type only localized tuberculosis was produced at the last test. The secondary guinea pig inoculated from the diseased tissues of this last test animal showed severe generalized tuberculosis, and cultures from the diseased tissues of both the original and the secondary test animal showed these germs to be active. No test made later than this showed any indication of live tubercle bacilli. Five such negative tests were made.

Series 2. Pure Cultures

Before the close of the first series it was recognized that these experiments were of such importance that a repetition of the same was advisable.

PREPARATION OF EMULSIONS

The cultures used and the preparation of the emulsions were similar to that in Series I.

Emulsion of the Human type.—The sample of water used for the emulsion of the human type was obtained from the pool June 15, 1911. With the sample was obtained also a considerable amount of floating green and yellow sediment scraped from the inside of the large tiling enclosing this pool of water. A microscopic examination showed very abundant algae—anabena, diatoms, desmids, scenedesmus, and confervoideae; also several kinds of bacteria. Among the animalcule there were vorticellae, amoeba, a few paramoecium, water-eels and rotifers.

There were thoroly shaken 450 cc. of this algal water to which was added an emulsion of approximately 5 mg. of human tubercle bacilli. This sample was divided into two equal portions: one portion was placed in an unglazed earthen, cylindrical jar eight inches tall and three inches in diameter; the other portion was placed in a small, unglazed vase which was stopped tightly with a one-hole rubber stopper in which was inserted a small glass tube that reached above the surface of the water and allowed the escape of any gases that might accumulate in the vase. This vase was placed on the bottom of the courtyard pool twenty inches below the surface of the water. The other sample in the 8-inch jar was placed on a slate shelf four inches below the surface of the water. This jar was open to the sunlight but the direct sun could not reach the surface of the emulsion inside. During the winter the part of this jar projecting above the surface of the water was crumbled by freezing. (See Fig. 1.)

Emulsion of the Bovine type.—The emulsion of the bovine type was made in a way similar to that of the human type. Five milligrams of these organisms from a young culture on glycerine egg were removed and rubbed up thoroly in a sample of 225 cc. of water obtained from the drinking trough at the dairy cattle barns, and thoroly mixed. A microscopical examination showed the following: (a) *vegetation*—spirogyra, oscillaria (large and small species, the small species being very abundant), a few diatoms, abundant protococcus, desmids, scenedesmus and anabena; (b) *animalcule*—amoeba, paramoecia, stylontia and vinegar eels.

This sample was placed along with the emulsion of the human type in a similar cylindrical jar on the slate shelf below the surface of the water in the pool.

RESULTS OF TESTS

The results of the tests of the bovine and the human types exposed in the open, unglazed, cylindrical jars are given in Tables 25 and 26. The last test in each, made 259 days since first exposed, showed only local tuberculosis in each of the four guinea pigs inoculated. Tho there was not time to determine by cultures and by secondary inoculations whether these organisms were living, it is likely that they were. The end point in this series was not reached. The tuberculosis produced in the test animals from all other samples, except those dying with acute infection, was severe and generalized. A typical autopsy is shown in the case of Guinea Pig 1124 (Fig. 3), which was inoculated with tubercle bacilli of the human type after exposure for 212 days in running water.

The results of the tests from the other part of the emulsion of human tubercle bacilli placed in the submerged vase are reported in Table 27. The last test, made on the 259th day, gave generalized tuberculosis in the two guinea pigs inoculated.

FIG. 3. GENERALIZED TUBERCULOSIS IN GUINEA PIG 1124 INOCULATED WITH HUMAN TUBERCLE BACILLI AFTER BEING EXPOSED IN RUNNING WATER FOR 212 DAYS.

TABLE 25.—DURATION OF LIFE OF BOVINE TUBERCLE BACILLI IN RUNNING WATER
(Experiment No. 2)

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	988	6–15–11	26	Killed	Generalized tuberculosis, severe	+	.....	....	.....	.....	.....
13	991	6–28–11	54	Killed	Generalized tuberculosis	+	+	1031	Tub. slight	+	+
43	1011	7–28–11	3	Died	Acute infection	+	?	1063	Gen. tub.	+	+
88	1058	9–11–11	3	Died	Acute infection and tuberculosis	....	....	.....	.....	.....	.....
91	1061	9–14–11	51	Killed	Generalized tuberculosis	+	+	1086	Gen. tub.	+	+
124	1076	10–17–11	42	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
152	1097	11–14–11	50	Died	Generalized tuberculosis	+	+	1122	Gen. tub.	+	+
173	1109	12– 5–11	48	Killed	Generalized tuberculosis, severe	+	+	1136	Tuberculosis	+	.....
212	1125	1–13–12	61	Died	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
259	1156	3– 1–12	89	Killed	Local tuberculosis R.S. inguinal	+	.....	.....	.....	.....	.....
259	1157	3– 1–12	89	Killed	Local tuberculosis R.S. inguinal	+	.....	.....	.....	.....	.....

TABLE 26.—DURATION OF LIFE OF HUMAN TUBERCLE BACILLI IN RUNNING WATER
(Experiment No. 2)

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	987	6–15–11	26	Killed	Generalized tuberculosis, severe	+	....	....	.....	.....	.....
13	992	6–28–11	54	Killed	Generalized tuberculosis	+	?	1030	Gen. tub.	+	+
43	1012	7–28–11	52	Killed	Generalized tuberculosis, severe	+	+	1062	Gen. tub.	+	+
88	1057	9–11–11	54	Killed	Generalized tuberculosis	+	+	1084	Gen. tub., severe	+	+
124	1075	10–17–11	4	Died	Acute infection	.....	....	....	.....	.....	.....
128	1081	10–21–11	25	Died	Acute infection	.....	....	...	.....	.....	.....
152	1096	11–14–11	46	Died	Acute infection, no tuberculosis	.....	.....	....	.....	.....	.....
162	1103	11–24–11	48	Died	Generalized tuberculosis	+	+	1123	Tub.	+	.....
173	1108	12–5–11	48	Killed	Generalized tuberculosis	+	+	1135	Tub.	+	.....
212	1124	1–13–12	89	Killed	Generalized tuberculosis, severe	+	+	1169	Gen. tub., severe	+	.....
259	1154	3–1–12	89	Killed	Tuberculosis, local	+	.....	....	.....	.....	.....
259	1155	3–1–12	89	Killed	Tuberculosis, local	+	.....	....	.....	.....	....

TABLE 27.—DURATION OF LIFE OF HUMAN TUBERCLE BACILLI IN A VASE SUBMERGED IN RUNNING WATER

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	988	6–15–11	26	Killed	Generalized tuberculosis, severe	+	.....	.....	.....	.....	.....
124	1078	10–17–11	42	Died	Generalized tuberculosis	+	.....	.....	.....	.....	.....
152	1099	11–14–11	20	Died	Acute infection, no tuberculosis	.....	.....	.....	.....	.....	.....
173	1111	12– 5–11	48	Killed	Generalized tuberculosis, severe	+	+	1137	Acute infection	.....	.....
212	1127	1–13–12	37	Died	Acute infection	.....	.....	.....	.....	.....	.....
259	1162	3– 1–12	89	Killed	Generalized tuberculosis	+	.....	.....	.....	.....	.....
259	1163	3– 1–12	89	Killed	Generalized tuberculosis	+	.....	.....	.....	.....	.....

The Diseased Tissues of a Tuberculous Guinea Pig

In order to test the length of time that tubercle bacilli will live when exposed in running water, Guinea Pig 918 was selected. This guinea pig, inoculated with tubercle bacilli of the human type, was killed 47 days after inoculation and found to be severely tuberculous. The autopsy showed the liver and the spleen to be much enlarged and thickly set with yellow masses of tubercles; the lungs and lymphatics were also extensively infected. Microscopical and cultural tests showed the tubercle bacilli to be characteristic and active.

MANNER OF EXPOSURE The dead tuberculous guinea pig was placed in a flower pot having the opening in the bottom closed. A half brick was placed upon the guinea pig to keep it it from floating up when placed in the running water of the pool. The flower pot was set on the bottom of the pool, which was twenty inches deep.

RESULTS OF TESTS The results are given in Table 28. All the tests made from the decaying and putrefying tissues of this guinea pig with one exception produced

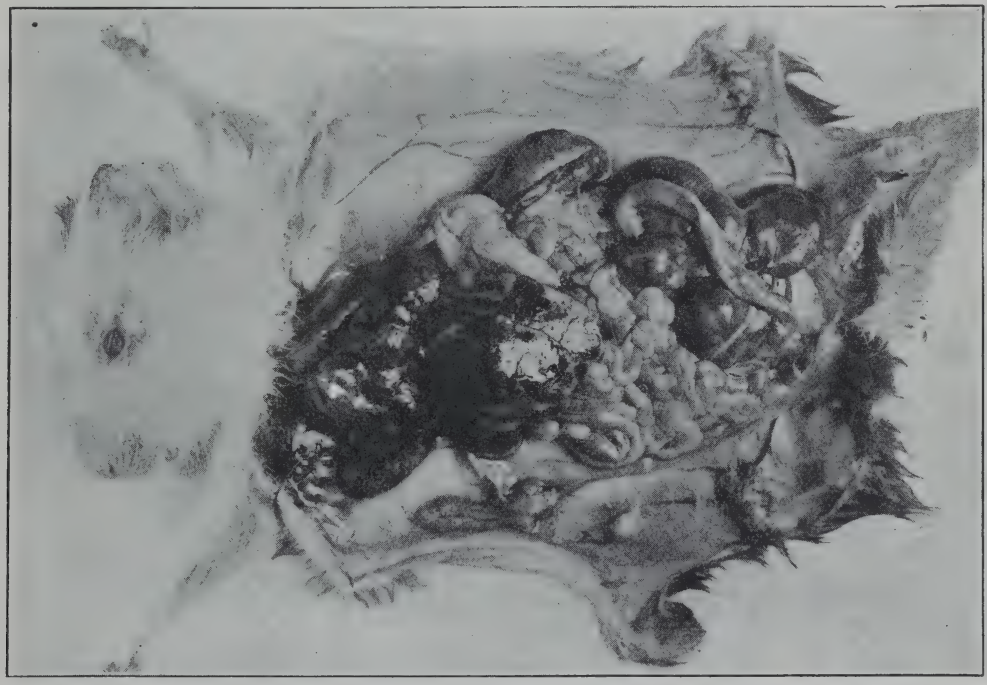

FIG. 4. GENERALIZED TUBERCULOSIS IN GUINEA PIG 1126 INOCULATED WITH TUBERCULOUS SPUTUM AFTER BEING EXPOSED IN RUNNING WATER FOR 187 DAYS.

TABLE 28.—DURATION OF LIFE OF HUMAN TUBERCLE BACILLI IN A DEAD GUINEA PIG KEPT IN RUNNING WATER

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	918	3- 2-11	47	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
70	958	5-11-11	75	Killed	Generalized tuberculosis, severe	+	+	1008	Gen. tub., severe	+	+
70	959	5-11-11	75	Killed	Generalized tuberculosis, severe	+	+	1008	Gen. tub., severe	+	+
99	983	6- 9-11	73	Killed	Healthy	.....	.....	.....	.....	.....	.....
99	984	6- 9-11	88	Killed	Healthy	.....	.....	.....	.....	.....	.....
134	999	7-13-11	43	Killed	Generalized tuberculosis, severe	+	+	1036	Gen. tub., severe	+	+
162	1022	8-11-11	13	Died	Acute infection	.....	.....	.....	.....	.....	.....
179	1044	8-28-11	67	Killed	Generalized tuberculosis, severe	+	+	1083	Gen. tub., severe	+	+
179	1045	8-28-11	67	Killed	Generalized tuberculosis, severe	+	+	1083	.....	.....	.....
193	1060	9-11-11	54	Killed	Generalized tuberculosis, severe	+	+	1085	Gen. tub., severe	+	+
229	1077	10-17-11	42	Died	Generalized tuberculosis, severe	+	+	1104	Gen. tub., severe	+	.....
257	1100	11-14-11	14	Died	Acute infection, no tubercles	.....	.....	.....	.....	.....	.....
278	1112	12- 5-11	48	Killed	Generalized tuberculosis, severe	+	+	1138	Tub.	+	.....
321	1128	1-17-12	44	Died	Tuberculous and acute infection	+	+	1146	Gen. tub., severe	+	.....
364	1158	3- 1-12	3	Died	Acute infection	.....	.....	.....	.....	.....	.....
364	1159	3- 1-12	3	Died	Acute infection	.....	.....	.....	.....	.....	.....
381	1165	3-18-12	36	Died	Acute infection	.....	.....	.....	.....	.....	.....

tuberculosis, save those that died too early with acute infection. The two guinea pigs used in the third test, made 99 days after exposure, remained healthy. Probably only non-tuberculous tissue was obtained for this test. Later tests taken from broken-down tissues showed virulent tubercle bacilli. The tests were positive up to the 321st day; after this date the test animals all died with acute infection, the last one, however, not too early to have shown tubercle bacilli. On the 229th day and later no structure of tissue could be determined except the hair and bones, and the bones were almost as fragile as garden earth. Sediment from the bottom of the flower pot was obtained for the samples.

Tuberculous Sputum

SOURCE OF SAMPLE To determine the length of time that tubercle bacilli from tuberculous sputum will live in running water, a sample was obtained from an advanced case of tuberculosis. A stained smear of this sputum showed numerous bacilli. A very small sample was inoculated into a guinea pig the first day of exposure. This guinea pig died in three days of acute infection.

RESULTS The results are given in Table 29. Tuberculosis has been produced in the test animals up to the last test, 232 days since exposure. However, only local tuberculosis was produced at this last test and there has not been sufficient time to determine whether the organisms are alive or dead. The test made just previous to the last one, 187 days after exposing the sputum in water, produced severe generalized tuberculosis in Guinea Pig No. 1126 (see Fig. 4).

TABLE 29. TUBERCLE BACILLI IN WATER

Kind and source of the organisms	Not killed	Killed
Human tubercle bacilli:		
a. Pure culture in flower pot, Series 1	441 days	470 days
b. Pure culture in 8-inch cylinder, Series 2	259 days	
c. Pure culture in submerged vase	259 days	
d. In tuberculous guinea pig	321 days	381 days
e. In sputum	232 days	
Bovine tubercle bacilli:		
a. Pure culture in flower pot, Series 1	441 days	470 days
b. Pure culture in 8-inch cylinder, Series 2	259 days	
c. Pure culture in cotton-stoppered bottle	202 days	

TABLE 30.—DURATION OF LIFE OF HUMAN TUBERCLE BACILLI FROM SPUTUM IN RUNNING WATER

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	996	7–10–11	3	Died	Acute infection	.....	.....	....	.....	.....	.....
39	1015	8–18–11	38	Killed	Generalized tuberculosis, severe	+	+	1064	Gen. tub., severe	+	+
63	1059	9–11–11	56	Killed	Generalized tuberculosis	+	+	1087	Gen. tub., severe	+	+
99	1074	10–17–11	42	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
127	1098	11–14–11	17	Died	Acute infection	.....	.....	.....	.....	.....	.....
156	1110	12–5–11	44	Died	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
187	1126	1–13–12	61	Died	Generalized tuberculosis, severe	+	+	1164	Tuberculous	+	.....
232	1160	3–1–12	47	Died	Acute infection	.....	.....	.....	.....	.....	.....
232	1161	3–1–12	89	Killed	Tuberculosis, local	+	.....	.....	.....	.....	.....

General Summary

A summary of the results showing the length of time that tubercle bacilli from various materials live in water is given in Table 29.

Conclusions

1. Tubercle bacilli live for more than a year (441 days) in running water.

2. The length of time that human and bovine tubercle bacilli live in water is practically the same.

3. These organisms live in sputum exposed in water for more than 232 days.

4. They live in the tissues of a dead guinea pig exposed in water for more than 321 days but are dead in 381 days.

5. A watering trough harboring tubercle bacilli may become a dangerous source of infection to cattle.

6. A dead tuberculous animal in a stream on your neighbor's farm may be a means of infecting your stock.

7. The better disposition of dead tuberculous animals is to destroy by burning.

8. Tubercle bacilli in drinking water is one of the possible sources of infection for man.

9. Infection is not prevented by dilution, since clumps containing a great number of these organisms may be inclosed in mucoid material which prevents their separation and destruction.

DURATION OF LIFE OF BOVINE TUBERCLE BACILLI IN BUTTER

INTRODUCTORY STATEMENT

Considerable attention is at present being directed to the presence of tubercle bacilli in foods, more especially milk, butter and cheese. It has been determined by Sedgwick and Winslow and by Park that typhoid bacilli frozen in water die very rapidly. After an hour's freezing 30 to 60 percent were destroyed, and in two weeks 99 percent were killed. The remaining one percent lived for a number of weeks. Tubercle bacilli in butter kept at a temperature below freezing are not killed in this way, as determined by Mohler, Washburn and Rogers in 1909. In order to obtain further information upon this subject the following experiments were planned.

Butter was mixed with an emulsion of a pure culture of bovine tubercle bacilli and placed in small vials which were stored in the three following places:

1. The cold storage of the Monarch Refrigerating Company, Chicago, Ill., at 10° C. below freezing.

2. The University of Illinois Dairy storeroom at 4° C. (above freezing).

3. The basement of a dwelling in Urbana, Illinois, kept at an average of approximately 20° C.

PREPARATION OF SAMPLES A pound of fresh butter was obtained from the creamery of the University of Illinois directly from the moulding board. It was salted as usual for the market, one ounce of dry salt to one pound of butter. After the butter is mixed, pressed and drained it has a salt content of two to three percent. It was not chilled, but at once taken to the bacterioligocal laboratory and mixed with the emulsion of tubercle bacilli. An emulsion of 3 mg. of bovine tubercle bacilli in 100 cc. of 0.8 percent salt solution was made, and the butter melted at 35° C. was thoroly shaken with this emulsion. From about 10 to 15 cc. of this emulsion were put into small sterile glass vials and stopped with sterile cork stoppers. Thirty such samples were prepared, ten of which were stored in each of the three places mentioned above.

TESTING THE SAMPLES The samples were tested when prepared and at varying intervals afterward on the same day from each of the places stored. They were brought to the laboratory, melted at a temperature of about 38° C., and two guinea pigs were each injected subcutaneously with 1 cc. of the melted butter from each sample. The samples kept at Chicago at 10° C. below freezing were always in excellent condition; those kept in the basement of the dwelling became very rancid and slightly mouldy; and those kept in the University Dairy storage showed slight moulding in part of the bottles.

RESULTS The results of the tests are given in Tables 31, 32 33. No end point was reached. Generalized tuberculosis was produced in the test animals from each of the three samples taken on the 274th day. It was noted that the tuberculosis produced by the samples kept at the lower temperature was more severe. This was probably due to the killing out of other organisms that at higher temperatures acted antagonistically to the tubercle bacilli.

TABLE 31.—DURATION OF LIFE OF BOVINE TUBERCLE BACILLI IN BUTTER AT —10° C.

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	1018	8–8–11	38	Killed	Generalized tuberculosis, severe	+	+	....	.....	.....	.....
0	1019	8–8–11	38	Killed	Generalized tuberculosis, severe	+	+	....	.....	.....	.....
17	1038	8–25–11	33	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	....
17	1039	8–25–11	38	Killed	Generalized tuberculosis, severe	+	....	.....	.....	.....	.....
34	1049	9–9–11	55	Killed	Generalized tuberculosis, severe	+	.....	.....	.....	.....	.....
34	1050	9–9–11	55	Killed	Generalized tuberculosis, severe	+	.....	.....	.....	.....	.....
59	1066	10–6–11	31	Killed	Generalized tuberculosis, severe	+	+	....	.....	.....	.....
59	1067	10–6–11	31	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
92	1088	11–8–11	38	Died	Generalized tuberculosis, severe	+	.....	.....	.....	.....	.....
93	1089	11–8–11	33	Died	Generalized tuberculosis, severe	+	....	.....	.....	.....	.....
128	1115	12–14–11	9	Died	Slight tub. and acute infection	+	....	.....	.....	.....	.....
128	1116	12–14–11	40	Killed	Generalized tuberculosis, severe	+	+	1139	Tuberculous	+	+
164	1129	1–19–12	40	Died	Tuberculous and acute infection	+	....	.....	.....	.....	.....
164	1130	1–19–12	40	Died	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
205	1148	3–1–12	38	Died	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
205	1149	3–1–12	42	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
274	1172	5–9–12	30	Killed	Generalized tuberculosis	+	....	....	.....	.....	.....
274	1173	5–9–12	30	Killed	Generalized tuberculosis	+	....	.....	.....	.....	.....

TABLE 32.—DURATION OF LIFE OF BOVINE TUBERCLE BACILLI IN BUTTER AT +4°C.

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	1018	8– 8–11	38	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
0	1019	8– 8–11	38	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
17	1040	8–25–11	33	Killed	Generalized tuberculosis, severe	+	+	....	.....	.....	.....
17	1041	8–25–11	33	Killed	Generalized tuberculosis, severe	+	.....	.....	.....	.....	.....
34	1051	9–11–11	53	Killed	Generalized tuberculosis, severe	+	....	....	.....	.....	.....
34	1052	9–11–11	53	Killed	Generalized tuberculosis, severe	+	....	.....	.....	.....	.....
59	1068	10– 6–11	31	Killed	Generalized tuberculosis, severe	+	+	....	.....	.....	.....
59	1069	10– 6–11	31	Killed	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
92	1090	11– 8–11	50	Died	Generalized tuberculosis, severe	+	....	.....	.....	.....	.....
92	1091	11– 8–11	2	Died	Acute infection	.....	....	....	.....	.....	.....
128	1117	12–14–11	40	Died	Slight tuberculosis and acute infec.	+	+	1140	Gen. tub.	+	.....
128	1118	12–14–11	40	Killed	Generalized tuberculosis, severe	+	+	1140	" "	+	.....
164	1131	1–19–12	34	Died	Slight tuberculosis and acute infec.	+	....	..	.....	.....	.....
164	1132	1–19–12	52	Died	Generalized tuberculosis, severe	+	+	.....	.....	.....	.....
205	1150	3– 1–12	42	Killed	Generalized tuberculosis, severe	+	+	....	.....	.....	.....
205	1151	3– 1–12	42	Killed	Generalized tuberculosis, severe	+	+	....	.....	.....	.....
274	1174	5– 9–12	3	Died	Acute infection	.....	....	....	.....	.....	.....
274	1175	5– 9–12	30	Killed	Generalized tuberculosis	+	....	....	.....	.....	.....

TABLE 33.—DURATION OF LIFE OF BOVINE TUBERCLE BACILLI IN BUTTER AT +25° C

Sample exposed, days	G. pig No.	Date of inoculation	Days since inoculation	Mode of death	Result of guinea pig test						
					Macroscopic	Microscopic	Culture	Inoculation of diseased tissue			
								G. pig No.	Macroscopic	Microscopic	Culture
0	1018	8–8–11	38	Killed	Generalized tuberculosis, severe	+	+				
0	1019	8–8–11	38	Killed	Generalized tuberculosis, severe	+	+				
17	1042	8–25–11	33	Killed	Generalized tuberculosis, severe	+					
17	1043	8–25–11	33	Killed	Generalized tuberculosis, severe	+					
34	1053	9–9–11	55	Killed	Generalized tuberculosis, severe	+					
34	1054	9–9–11	55	Killed	Generalized tuberculosis, severe	+					
59	1070	10–6–11	31	Killed	Generalized tuberculosis, severe	+	+				
59	1071	10–6–11	31	Killed	Generalized tuberculosis, severe	+	+				
92	1092	11–8–11	26	Died	Tuberculosis and acute infection	+					
92	1093	11–8–11	24	Died	Tuberculosis and acute infection	+					
128	1119	12–14–11	40	Killed	Generalized tuberculosis, severe	+	+	1141	Tub.	+	
128	1120	12–14–11	40	Killed	Generalized tuberculosis, severe	+	+	1141	Tub.	+	
164	1133	1–19–12	31	Died	Acute infection, no tubercles						
164	1134	1–19–12	32	Died	Acute infection, no tubercles						
205	1152	3–1–12	42	Killed	Generalized tuberculosis	+	+	1170	Gen. tub.	+	
205	1153	3–1–12	42	Killed	Generalized tuberculosis	+	+	1170	Gen. tub.	+	
274	1176	5–9–12	30	Killed	Generalized tuberculosis	+					
274	1177	5–9–12	30	Killed	Generalized tuberculosis	+					

GENERAL DISCUSSION

A summary of the results of the experimental work is given in Table 34. It is seen from this table that tubercle bacilli in pure culture, spread in thin layers on sterile glazed paper slips and exposed to the direct rays of the sun, are killed in a very short time (1 to 4 minutes). In this respect tubercle bacilli may be classed with other nonspore-bearing organisms. When exposed to desiccation, pure cultures of these germs in thin layers are found to be dead in a few days. In sputum and other foul material they appear to live longer than the other nonspore-bearers. They are known to live long enough to be blown around so that the inhalation of dried sputum dust causes tuberculosis in test animals. Just how frequently people are infected by breathing dried tuberculous material has been discussed (see page 270). That sunlight plays an important part in the disinfection of this dried tuberculous dust is evident. Also that our homes, factories, and places of business should have an abundance of window space, located so as to admit the light, is another timely lesson.

Tubercle bacilli in cow manure lived 73 days when a pure culture mixed in a sample of manure was exposed to weather conditions in a pasture field in the shade, and as long as 49 days when exposed in the sunshine. The sample from a tuberculous cow was dead at the first test, made 13 days after exposure. We were disappointed in not being able to repeat these experiments. It would be advisable to repeat this work, especially with naturally infected manure from several tuberculous cows that were known to be expelling tubercle bacilli per rectum. Keep pigs from three to four months old in a pasture with such tuberculous cows, and allow the pigs to feed upon the cow dung. Feed other pigs by mixing with their feed tuberculous manure which had remained in this pasture for varying intervals of time. An examination of the internal organs of these shoats would give valuable information both as to the infectiveness of the cow manure and to the length of time that tubercle bacilli remain alive in such manure. This would indicate, much more completely than our experiments, the length of time that stock should be kept from a field in which tuberculous cattle had been pastured.

When tubercle bacilli, either in manure or in dead tuberculous animals, become mixed in the soil, the danger may be still greater, depending upon the opportunity for hogs to take this material along with their food. The bacilli live longer under these conditions, but the opportunity of being taken is usually less.

The danger of man becoming infected with tuberculosis from drinking water has been discussed (see page 307). Just how likely

TABLE 34.—SUMMARY OF RESULTS

Organisms exposed to direct sunlight

Name of organism	Not killed	Killed
B. subtilis, spores	180 min.	
B. mesentericus vulgatus, spores		
B. subtilis, vegetative cells	½ min.	1 min.
B. mesenteric, vegetative cells		
B. prodigiosus		½ min.
B. diphtheria	6 min.	after 6 min.
B. coli	2 min.	3 min.
B. typhosus	1 min.	2 min.
B. violaceus	2 min.	3 min.

Tubercle Bacilli Exposed to Direct Sunlight

Name of culture	Not killed	Killed
B. tuberculosis, human	1 min.	2 min.
B. tuberculosis, bovine	1 min.	2 min.
B. tuberculosis, avian	2 min.	4 min.

Bovine Tubercle Bacilli in Cow Manure

Kind and source of material exposed	Not killed	Killed
Bovine tubercle bacilli, pure culture:		
Exposed in cow manure, in sunshine, Series 1.	49 days	81 days
Exposed in cow manure, in sunshine, Series 2.	45 days	73 days
Exposed in cow manure, in shade, Series 1	49 days	81 days
Exposed in cow manure, in shade, Series 2	73 days	100 days

Bovine Tubercle Bacilli from a Naturally Tuberculous Cow

Tuberculous manure from this cow, in sunshine.		13 days
Tuberculous manure from this cow, in shade		43 days

Bovine Tubercle Bacilli in Garden Soil

Bovine tubercle bacilli, pure culture	213 days	230 days
Bovine tubercle bacilli, in tissue of a dead guinea pig	77 days	91 days

Tubercle Bacilli in Water

Human tubercle bacilli:		
Pure culture, in flower pot, Series 1	441 days	470 days
Pure culture, in 8-inch cylinder	258 days	
Pure culture, in an unglazed vase, submerged.	259 days	
In the tissues of a tuberculous guinea pig	321 days	381 days
In tuberculous sputum	232 days	
Bovine tubercle bacilli:		
Pure culture in flower pot, Series 1	441 days	470 days
Pure culture, in an 8-inch cylinder	259 days	
Pure culture, in a cotton-stoppered bottle	202 days	

Bovine Tubercle Bacilli in Market Butter

Temperature stored	Not killed	Killed
10° C. below zero	274 days	
4° C. above zero	274 days	
20° C. above zero	274 days	

it is that cattle and other farm animals are infected with tuberculosis from the presence of these germs in water is not surely known. The common watering tank may become a source of infection. Here the tubercle bacilli live among the algae and in the decaying organic matter for more than a year. Calmette points out that constant and repeated infections are the most dangerous. Cattle would thus be subjected when a watering trough was infected.

Another source of danger to man is in the use of phosphates made by grinding up dead tuberculous animals (which is done rather extensively in the United States), this fertilizer often being used in vegetable gardening. One can easily conceive how a small piece of tuberculous tissue containing many dozens of tuberculous germs could be made to adhere to an onion or a radish, especially in a slightly bruised place, and be carried directly to the consumer. That these germs would remain alive and virulent during such a circuit there is no question.

It is seen that tubercle bacilli in butter kept at 10° C. below zero retain their virulence longer than when kept at the higher temperature. This temperature of —10° C. apparently has no injurious effect on these germs, while the antagonism of other organisms is largely prevented. Butter can be kept in cold storage for months in an excellent condition, but this in no way lessens the danger from tubercle bacilli that were originally introduced into the butter. All such dairy products should be tested by government officials not only for quality but also for the presence of tubercle bacilli.

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